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Comparison of Ames 'Salmonella Typhimurium' Plate Incorporation Test Protocols

Northrop Services, Inc.
Research Triangle Park, NC

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COMPARISON OF AMES *SALMONELLA TYPHIMURIUM* PLATE INCORPORATION TEST PROTOCOLS

by

Stephanie Toney
Northrop Services, Inc. - Environmental Sciences
Research Triangle Park, NC 27709

and

Larry D. Claxton
Genetic Toxicology Division
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

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Project Officer

Larry D. Claxton
Genetic Toxicology Division
Health Effects Research Laboratory
Research Triangle Park, NC 27711

This study was conducted
in cooperation with

Northrop Services, Inc. - Environmental Sciences
Research Triangle Park, NC 27709

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

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16. ABSTRACT

The purpose of this document is to tabulate the differences between the submitted protocols and to note the modifications fron the 1975 Ames reference. The tables show the differences obtained between laboratories in bacterial strain maintenance, metabolic activation systems, chemical controls, preparation of medias and solutions, and of the assay procedure itself. The recommendations of de Serres and Shelby and any revisions by Ames are also included.

No attempt is made to evaluate any laboratory or indicate a preferable method for the test. Some laboratories may have Standard Operating Procedures (SOP's) that are more detailed than the submitted protocols. Changes or revisions that have taken place in a protocol since submission are not noted in this document. In addition, laboratories that use Ames' reference (1975) as their main protocol may have only sent their modifications.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, North Carolina conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis, and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The reasons for this comparison of 25 protocols for Salmonella mutagenicity testing is three-fold. It serves as a reference for genetic toxicologists, as a starting point for creation of reference protocols for those who need precise Ames test protocols, and as a guide in understanding variations in test results.

F. G. Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

This document is a companion volume to the Compilation of Ames Salmonella typhimurium Plates Incorporation Test Protocols. The companion document compiled 25 protocols that were requested from various major laboratories across the country.

The purpose of this publication is to tabulate the differences between the submitted protocols and to note the modifications from the 1975 Ames reference. The tables show the differences obtained between laboratories in bacterial strain maintenance, metabolic activation systems, chemical controls, preparation of medias and solutions, and of the assay procedure itself. The recommendations of de Serres and Shelby and any revisions by Ames are also included.

No attempt is made to evaluate any laboratory or indicate a preferable method for the test. Some laboratories may have Standard Operating Procedures (SOP's) that are more detailed than the submitted protocols. Changes or revisions that have taken place in a protocol since submission are not noted in this document. In addition, laboratories that use Ames' reference (1975) as their main protocol may have only sent their modifications.

The authors feel that this comparison and its companion volume are a useful reference in writing better protocols and gaining more understanding of the variations of Ames test results from different laboratories.

CONTENTS

Disclaimer ii
Foreword iii
Abstract iv
Figures vii
Tables viii
Acknowledgment x

1. Introduction 1

2. Description of the Ames Salmonella typhimurium Plate Incorporation Test 7

3. Bacterial Strains 8
   Bacterial Strains Routinely Used 8
   Storage of Strains 9
   Histidine Requirement Test 9
   Determination of rfa Character 9
   Ampicillin Resistance 10
   uvrB Deletion 10
   Compound Sensitivity Verification 11
   Spontaneous Reversion Rate 11
4. Metabolic Activation System 25
   Preparation of S-9 fraction:
   I. Induction of Animal 25
   Preparation of S-9 fraction:
   II. Preparation of fraction 25
   Storage of S-9 fraction 26
   Preparation of S-9 mix 26
5. Stock Solutions 42
   Sodium Phosphate Buffer 42
   Microsomal Salt Solution 42
   Glucose Stock Solution 42
   Histidine/Biotin Stock Solution 43
   VBME Salt Solution 43
6. Media 60
   Top Agar Overlays 60
   Petri Plate Agar 60
   Nutrient Broth 60
7. Chemical Controls 68
   Control Mutagens/Carcinogens Routinely Used 68
   Preparation of Control Mutagens/Carcinogens 69

(Continued)

CONTENTS (Continued)

8. Assay Procedure 76
9. Safety Precautions 81
10. Discussion 82

Bibliography 85

FIGURES

Number		Page
1	Schematic for plate incorporation test	3
2	Initial letter of request	4
3	Follow-up letter of request	5

TABLES

Number		Page
1	Names and Addresses of Participating Laboratories	6
2	Bacterial Strains Used in Routine Testing	8
3	Histidine Requirement Test for Bacterial Strains	12
4	Determination of rfa Character in Bacterial Strains	16
5	Ampicillin Resistance Test for Bacterial Strains	19
6	Determination of uvrB Deletion Mutation in Bacterial Strains	22
7	Preparation of S-9 Fraction: I. Induction of Animal	27
8	Preparation of S-9 Fraction: II. Preparation of Fraction	31
9	Storage of S-9 Fraction	35
10	Preparation of S-9 Mix	38
11	Sodium Phosphate Buffer Preparation	44
12	Microsomal Salt Solution Preparation	47
13	Glucose Stock Solution	51
14	Histidine/Biotin Stock Solution	54
15	Vogel Bonner Medium E Salt Solution	58
16	Top Agar Overlay Preparation	61
17	Petri Plate Agar	64
18	Nutrient Broth Preparation	67
19	Control Mutagens/Carcinogens Routinely Used	70

(Continued)

TABLES (Continued)

Number		Page
20	Preparation of Control Mutagens/Carcinogens	74
21	Assay Procedure	77
22	Inclusion of Procedures and Preparation in 25 Ames *Salmonella* Plate Incorporation Protocols	84

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The authors would like to thank the members of each laboratory who responded to our request for the submission of a protocol. We would also like to acknowledge the assistance of Ms. Carole Moussalli of Northrop Services, Inc., Research Triangle Park, NC, for her editorial assistance.

SECTION 1

INTRODUCTION

Bacterial tests for mutagenicity have been available for approximately 30 years. In 1951, Demarec, et al., found that 19 out of 31 chemicals tested were mutagenic using an Escherichia coli reverse mutation system. After modifying the mutation system described by Demarec, Szybalski (1958) tested 431 substances for mutagenicity. Recognizing the inadequancies of the streptomycin resistance and the α-azetidine carbolic acid resistance systems, Ames (1971) published a method for a bacterial system using a histidine-requiring mutant of Salmonella typhimurium. Malling (1971) was the first to merge a mammalian metabolizing system with a bacterial system in order to demonstrate the mutagenicity of dimethylnitrosamine. In 1975, Ames et al., published a detailed protocol that incorporated the use of a mammalian metabolizing system, thereby establishing the "Ames test" shown in Figure 1 as a routine screening system for mutagenicity and potential carcinogenicity. Although the 1975 paper by Ames, et al., provided a highly detailed protocol, many laboratories have introduced a variety of changes and/or additions to this standard protocol. In addition, summaries of four international meetings/workshops have been published (Mattern and Greim, 1978; Seiler et al., 1979; de Serres and Shelby, 1979a; de Serres and Shelby, 1979b; Stich and San, 1979).

Since so many researchers have published suggested alterations and additions to the original protocol, the U.S. Environmental Protection Agency (EPA) decided to conduct an informal survey of laboratories that utilize the Ames test. A list of laboratories performing the Ames test in a routine manner was obtained from Dr. Mike Shelby at the National Institute of Environmental Health Sciences, Research Triangle Park, NC. An initial letter reprinted in Figure 2 was sent to the 46 laboratories included in this list. The follow-up letter in Figure 3 was sent to the laboratories who did not respond within a one-month interval. Thirty-three laboratories responded to the request. The respondents included six laboratories that no longer performed the Ames test, one laboratory which had closed and 25 laboratories which submitted protocols. The other responding laboratory elected to restrict usage of their protocol. A list of names and addresses of participating laboratories is found in Table 1. A companion publication, "Compilation of Ames Salmonella typhimurium Plate Incorporated Test Protocol", is a simple compilation of the protocols and cover letters submitted. This publication is a tabulated qualitative summary and comparison of the submitted protocols. The compilers and the EPA understand that these protocols may not accurately reflect the competence or performance of any laboratory. Some laboratories may maintain Standard Operating Procedures (SOP's) that are more detailed than the submitted protocols and some may have modified and/or expanded their procedures since submission of these

documents. Inclusion in this volume is neither an attempt to criticize nor endorse a given procedure or method. However, the compilers believe that this comparison and its companion compilation are useful references, guides for writing better protocols, and vehicles in understanding the variations in results among laboratories.

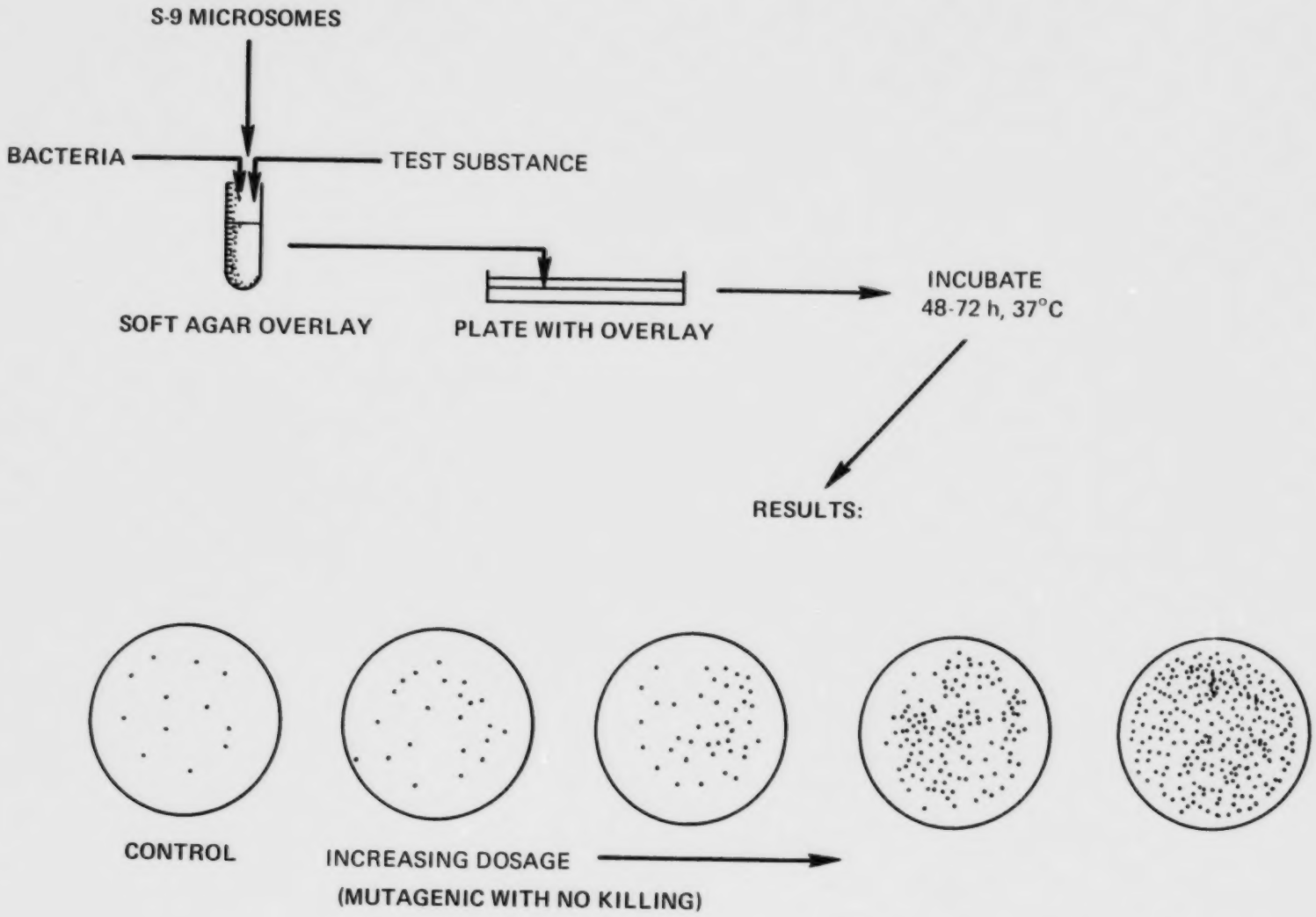

Figure 1. Schematic diagram of plate incorporation test.

November 26, 1980

Dear

Since you head a well-known laboratory in the Ames Salmonella assay I would like to request a current Ames Test Protocol from you. I am working for Dr. Larry Claxton in preparing a compilation of Ames test protocols for the Environmental Protection Agency. This compilation will serve several purposes:

(1) A reference for workers within the genetic toxicology field,

(2) a starting point for the creation of reference protocols for EPA laboratories, industrial laboratories, and others who have a need for precise Ames test protocols, and

(3) as a guide to understanding variations in Ames test results.

Your protocol is needed in order to document differences between protocols in this compilation.

A final copy of the compilation will be sent to you if you would like one.

Please address the protocol to:

Stephanie Toney
USEPA, HERL, GTD, GBB (MD-68)
Research Triangle Park, NC 27711

Thank you for your prompt consideration.

Sincerely yours,

Stephanie Toney
Genetic Bioassay Branch (MD 68)

Figure 2. Initial letter sent to laboratories.

January 16, 1981

Dear

Prior to Thanksgiving an Ames Test Protocol was requested from your laboratory. In the holiday rush, this letter may have been overlooked. If you have sent the protocol recently, please disregard this request.

Your protocol is desired for a compilation being completed in collaboration with Dr. Larry Claxton of the Environmental Protection Agency. This compilation will serve several purposes:

(1) A reference for workers within the genetic toxicology field,

(2) a starting point for the creation of reference protocols for EPA laboratories, industrial laboratories, and others who have a need for precise Ames test protocols, and

(3) as a guide to understanding variations in Ames test results.

Your protocol will be use to document differences between protocols in this compilation.

A final copy of the compilation will be sent to those laboratories participating in the study.

Please address the protocol to:

Stephanie Toney
USEPA, HERL, GTD, GBB (MD-68)
Research Triangle Park, NC 27711

Thank you for your prompt consideration.

Sincerely yours,

Stephanie Toney

Figure 3. Follow-up letter sent to laboratories.

TABLE 1. NAMES AND ADDRESSES OF PARTICIPATING LABORATORIES

Dr. Andrew Sivak
Arthur D. Little, Inc.
25 Acorn Park
Cambridge, MA 02140

Mr. James P. Crowley
Battelle Columbus Laboratories
505 King Avenue
Columbus, OH 43201

Dr. Douglas M. Hanson
Bioassay Systems Corporation
100 Inman Street
Cambridge, MA 02139

Mr. Clyde R. Goodheart
Biolabs, Inc.
2910 MacArthur Blvd.
Northbrook, IL 60062

Dr. Rae E. Drazin
Bio-Technics Laboratories, Inc.
1133 Crenshaw Boulevard
Los Angeles, CA 90019

Dr. William T. Speck
Case Western Reserve University
Department of Pediatrics
2103 Adelbert Road
Cleveland, OH 44106

Dr. Steve Haworth
EG&G Mason Research Institute
1530 E. Jefferson Street
Rockville, MD 20852

Dr. Larry Claxton
Genetic Toxicology Division
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Dr. Vincent F. Simmon
Genex Corporation
6110 Executive Blvd.
Suite 1090
Rockville, MD 20852

Ms. Nancy E. McCarroll
Hazleton Laboratories America, Inc.
9200 Leesburg Turnpike
Vienna, VA 22180

Dr. George C. Lavelle
Hill Top Research, Inc.
Department of Toxicology
Miamiville, OH 45147

Mr. Peter W. Barbera
IIT Research Institute
Life Sciences Division
10 West 35th Street
Chicago, IL 60616

Ms. Shirley Louie
Jefferson Professional Services
P.O. Box 3397
Little Rock, AR 72203

Dr. Andrew M. Tometsko
Litron Laboratories, Ltd.
1351 Mt. Hope Avenue
Rochester, NY 14620

Dr. David Brusick
Litton Bionetics, Inc.
5516 Nicholson Lane
Kensington, MD 20795

Dr. Carol Richardson
Meloy Laboratories, Inc.
6715 Electronic Drive
Springfield, VA 22151

Mr. Andrew M. Losinkoff
Microbiological Associates
5221 River Road
Bethesda, MD 20016

Ms. JoAnne Gridley
Monsanto Research Corp.
1515 Nicholas Road
Dayton Laboratory
Dayton, OH 45407

Dr. John E. Preston
USEPA-NEIC
Bldg. 53
Box 25227
Denver, CO 80225

Dr. Bruce C. Casto
Northrop Services, Inc.
P.O. Box 12313
Research Triangle Park, NC 27709

Dr. Robert A. Finch
Raltech Scientific Services
A Division of Ralston Purina Co.
P.O. Box 7545
Madison, WI 53707

Mr. Thomas J. Hughes
Research Triangle Institute
Box 12194
Research Triangle Park, NC 27709

Dr. Nathan Greene
Southwest Foundation for Res. & Ed.
P.O. Box 23147
San Antonio, TX 78284

Dr. David C.L. Jones
SRI International
333 Ravenswood Avenue
Menlo Park, CA 94025

Ms. Melanie Baltezore
UNILAB Research
2800 7th Street
Berkeley, CA 94710

SECTION 2

DESCRIPTION OF THE AMES SALMONELLA TYPHIMURIUM PLATE INCORPORATION TEST

The Ames Test is a short term bioassay for mutagenicity testing. The advantages of this test are the speed with which results can be obtained, usually 3-4 days, and its relatively low cost. The Ames Test is used for testing both pure chemicals (cyclophosphamide and 6-mercaptopurine) and complex mixtures, including mobile source emissions (diesel, gasoline) and comparative source emissions (coke oven, roofing tar, cigarette smoke condensate).

In the Ames Test, a given sample is added to a broth culture of Salmonella in an agar overlay tube which is then plated on minimal media as shown in Figure 1. The sample is routinely tested at several dose levels with five Ames strains of Salmonella with and without metabolic activation. The dose levels are plated usually in duplicate or triplicate, a process involving approximately 120 plates per sample excluding control plates. After the bacteria, test compound, and possibly metabolic activator are added to the overlay tube the contents are gently mixed and plated out on Vogel-Bonner minimal media plates. These plates are incubated for two or three days at 37°C in the dark. The plates are then scored for mutants.

There are five Ames strains in widespread use: TA1537, TA1538 and its derivative TA98, and TA1535 and its derivative TA100. These strains are all histidine-dependent mutants and revert to wild type in the Ames Test. Two of the strains detect frameshift mutations (TA1537, TA1538) and one strain detects basic pair substitutions (TA1535). Strains TA98 and TA100 are less specific in detecting the mutagen type. TA98 and TA100 both contain an R factor plasmid which increases the sensitivity of these strains. All five strains have an rfa mutation creating a deficiency in the cell wall, and, therefore, increasing permeability to macromolecules. The strains all have a uvrB mutation which decreases genetic repair ability.

The mammalian metabolic activation system is added to the overlay in order to identify compounds that require metabolic activation mechanisms of mammals not found in bacteria. The metabolic activation system permits metabolites of the test compound to be tested for mutagenicity. The activation system consists of a 9000xg supernatant (S-9) of Aroclor 1254 induced rat liver homogenates.

SECTION 3

BACTERIAL STRAINS

BACTERIAL STRAINS ROUTINELY USED

The Ames reference (1975) suggested the use of three standard strains for routine testing (TA1535, TA1537, and TA1538) to be used in combination with the newer derivative strains (TA98, TA100). Ames allowed for the possibility that TA1538 be omitted in favor of the more sensitive strain TA98. de Serres and Shelby recommended that TA1538 not be omitted in a routine screening test.

The majority of the participating laboratories use all five tester strains routinely. Two of the laboratories shown in Table 2 do not use TA1538 routinely. One uses only three strains: TA98, TA100, and TA1537. Another does not say what strains it uses. One laboratory appears to substitute TA1575 for TA1535; however, this may be a typographical error.

TABLE 2. BACTERIAL STRAINS USED IN ROUTINE TESTING: LABORATORIES WITH RECOMMENDATIONS DIFFERING FROM REFERENCE PAPERS[a]

Laboratory	Strains Used
Arthur D. Little	TA98, TA100, TA1535, TA1537
Biolabs[b]	TA98, TA100, TA1575, TA1537, TA1538
Case Western	no data available
Monsanto[c]	TA98, TA100, TA1535, TA1537
UNILABS	TA98, TA100, TA1537

[a]All laboratories not listed use strains recommended by Ames, et al. and de Serres and Shelby. (TA98, TA100, TA1535, TA1537, TA1538).
[b]The TA1575 may be a typographical error.
[c]TA1538 is used when specified by the client.

STORAGE OF STRAINS

Only Ames discussed soft agar for storage of strains. He uses 2 g Difco-Bacto Nutrient Broth and 1.5 g agar to 250 ml H_2O. A quantity of 1.2 ml is dispensed in a 2 ml sterile glass vial with a rubber-lined screw cap, that is then sealed with paraffin and stored at room temperature.

Ames altered the 1975 reference and is now employing master plates for the temporary storage of strains. His laboratory stores master plates in a cold room; all culturing from these plates is done in the cold room. The master plates are kept for a period of one to two months. Their preparation involves making minimal glucose agar and spreading histidine and biotin onto these plates. Ampicillin is also spread on plates used for strains TA98 and TA100. These plates sit for one or two days before use to allow the compounds to diffuse into the media.

The six laboratories that list a procedure report the same procedure as Ames. Permanent storage is in 2 ml sterile glass vial with rubber-lined screw tops. The culture is stored at a concentration of 0.8 ml of a 16 h nutrient broth culture to 0.07 ml DMSO. It is kept at -80°C in a Revco freezer. The laboratories that list a procedure agree with the Ames permanent test and revised temporary storage procedures.

HISTIDINE REQUIREMENT TEST

Ames gave very specific instructions for checking out tester strains periodically. His disussion included procedures for monitoring histidine requirement, determination of rfa character retention, ampicillin resistance in TA98 and TA100, increased mutagenicity of strains TA98 and TA100 over TA1538 and TA1535, presence of the uvrB deletion, and spontaneous reversion rate.

Ames' recommendations for the histidine requirement test specify minimal media petri plates. The Salmonella is streaked onto the plate. An aliquot of 0.1 ml of a 0.1 M solution of L-histidine is spread onto the plate. Biotin is also used with strains that have the uvrB deletion. An aliquot of 0.1 ml of a 0.5 mM solution is spread onto the plates. Plates without histidine are used as a negative control.

For the histidine requirement test, nine laboratories do not mention the methods they employ to monitor this requirement as shown in Table 3. Of the ones who mentioned a procedure, five laboratories say they check this requirement but give no description. Nine laboratories repeat the Ames procedure in their protocol. Two others use an overlay rather than streak the bacteria on the plate.

DETERMINATION OF rfa CHARACTER

Ames used nutrient agar plates and a 16-h incubation of Salmonella in nutrient broth culture. The Salmonella should be at a 10^8 concentration. A total of 0.1 ml of the tester strain is added to an agar overlay and poured on the nutrient agar plate. Ames gives 2 possibilities for a sensitivity test compound: an aliquot of 10 µl of a 1 mg/ml solution of crystal violet or 2 mg sodium deoxycholate. The test compound is applied to a sterile filter paper

disc which is then placed on the petri plate. The plate is incubated for 12 h at 37°C and then interpreted. If the mutation is still present there will be a zone of inhibition around the disc. The size of the zone is approximately 14 mm for crystal violet and 13 mm for deoxycholate.

Of the participating laboratories presented in Table 4, six do not list a method of determination, and four mention that they do the procedure in their laboratory. Of the remaining laboratories, nine repeat Ames' procedure, and two use minimal agar rather than nutrient agar. One laboratory mentions the compound and add that they check for this requirement. One laboratory streaks the Salmonella rather than use an overlay. Two others use Mueller-Hinton plates in combination with a swab-streak of Salmonella.

AMPICILLIN RESISTANCE

Ames (1975) recommended using nutrient agar plates for the ampicillin resistance test. A total of 10 µl of an 8 mg/ml ampicillin solution in 0.02 N NaOH is streaked on the plate and cross-streaked with Salmonella. After 12-24 h of incubation at 37°C, the plates are evaluated. If the mutation is present (TA98, TA100) there should be no zone of inhibition around the streak.

de Serres and Shelby recommended that the ampicillin be put on the plate in a disc form and used in combination with a top-agar overlay.

Six of the laboratories do not list a procedure for this test as shown in Table 5. Six others say they perform this test but give no instructions.

Three laboratories follow the procedure given in Ames 1975. Four laboratories follow the recommendation of de Serres and Shelby. Two use a Mueller-Hinton plate with an Ampicillin disc and a swab-streak of Salmonella. Three others spread the ampicillin over the entire plate and score the mutation as present with growth on the plate. One laboratory does not check for this mutation.

uvrB DELETION

Ames' procedure for this test uses nutrient broth agar with a streak of Salmonella on the plate. One half of the plate is irradiated with a UV light for 6 seconds (8 seconds for TA98, TA100) at a distance of 33 cm (i.e., plate to lamp distance). The plate is then incubated for 12-24 h at 37°C and scored for the presence of the mutation. If the mutation is present no growth will occur on the exposed side.

Eight laboratories perform this test as described in Table 6. Eight do not list a procedure, and four say they perform the test. One differs from Ames only in using Mueller-Hinton plates. Two laboratories use an overlay rather than a streak for the Salmonella and also use different time intervals. One laboratory irradiates sections of the plate from 10 sec to one minute, and incubates for 48 h. One laboratory does not list a procedure but say they perform Ames' identically.

COMPOUND SENSITIVITY VERIFICATION

Ames recommended comparing TA98 and TA100 to their parent strains in a standard assay with Aflatoxin B_1 and methyl methanesulfonate to show increased mutagenesis by TA98 or TA100. Only one laboratory mentions this procedure.

SPONTANEOUS REVERSION RATE

Ames suggested that the spontaneous reversion rate be checked in a standard assay; an acceptable interpretation is that plate counts fall within the standard individual laboratories limits. Five laboratories check the reversion rate but give no specifics on their operating procedure. Eleven others follow the Ames suggestion and nine do not mention the procedure.

TABLE 3. HISTIDINE REQUIREMENT TEST FOR BACTERIAL STRAINS

Specifications	Reference papers			Laboratories[a,b]		
	Ames	de Serres	Ames revised	Biolabs	Biotechnics	Genetic Bioassay Branch
Media for test	minimal plates			minimal agar plates	minimal agar plates	minimal media
Salmonella test culture	streak on plate					nutrient broth culture overlay
L-Histidine concentration	sterile 0.1 M	no data available	no data available	sterile 0.1 M	sterile 0.1 M	0.1 M
Quantity of histidine	0.1 ml/plate			0.1 ml/plate		0.1 ml in overlay
Negative control	no histidine added			no histidine added	no histidine added	no histidine added
Use of biotin	with uvrB deletion strains			all strains	with uvrB deletion	all strains
Biotin concentration	0.5 mM			0.5 mM	0.5 mM	0.5 mM
Quantity of biotin	0.1 ml/plate			0.1 ml/plate		0.1 ml in overlay

(Continued)

TABLE 3. (Continued)

Specifications	Laboratories		
	IITRI	Monsanto	Meloy
Media for test	minimal agar plates	minimal agar plates	minimal agar with 0.1 ml His/Bio spread on surface
Salmonella test culture	streak	streak	streak
L-Histidine concentration		0.1 M	
Quantity of histidine		0.1 ml spread on plate	
Negative control	no histidine added	no histidine added	no His/Bio
Use of biotin	all strains		
Biotin concentration	0.5 mM	0.5 mM	
Quantity of biotin	0.1 ml	0.1 ml spread on plate	

(Continued)

TABLE 3. (Continued)

Specifications	Laboratories		
	NEIC-EPA	Northrop	Raltech
Media for test	minimal plates	minimal agar media	minimal glucose agar
Salmonella test culture	cross-streak	streak	streak
L-Histidine concentration	0.1 M	0.1 M	0.1 M
Quantity of histidine	0.1 ml	0.1 ml spread on plate	100 µl His/Bio
Negative control	no histidine added	no histidine added	no histidine added
Use of biotin			
Biotin concentration	0.5 mM		0.5 mM
Quantity of biotin	0.1 ml		

(Continued)

TABLE 3. (Continued)

Specifications	Laboratories	
	RTI	Southwest
Media for test	histidine deficient media	minimal media
Salmonella test culture	Oxoid broth culture overlay	streak
L-Histidine concentration	48 mg/l H_2O in overlay and media	1 g/100 ml H_2O
Quantity of histidine		0.1 ml spread on plate
Negative control	histidine negative overlays	
Use of biotin		
Biotin concentration		10 mg/100 ml H_2O
Quantity of biotin		0.1 ml spread on plate

[a]The following laboratories do not list a procedure: Arthur D. Little, Battelle, Bioassay Systems, Case Western, EG&G Mason, Jefferson, Litton, Microbiological Assoc., Unilab.

[b]The following laboratories check this requirement but give no description: Genex, Hazleton, Hilltop, Litron, SRI.

TABLE 4. DETERMINATION OF rfa CHARACTER IN BACTERIAL STRAINS

Specifications	Reference papers			Laboratories[a,b]		
	Ames	de Serres	Ames revised	Bioassay Systems	Biolabs	Biotechnics
Media for test	nutrient agar plates			Mueller-Hinton plate	nutrient agar	nutrient agar
Salmonella test culture	16 h nutrient broth culture				12 h culture	overnight broth culture
Quantity of *Salmonella*	0.1 ml of 10^8 concentration culture				10^8 concentration culture	0.1 ml x 10^8 concentration
Method of *Salmonella* addition	top agar overlay	no data available	no data available	swab-streak		overlay
Compound for sensitivity test	crystal violet and/or sodium deoxycholate			crystal violet	crystal violet	crystal violet
Method of application for compound	paper disc on middle of plate			disc at end of streak	disc	disc
Compound concentration	crystal violet 10 µl of 1 mg/ml 2 mg deoxycholate			10 µl of 1 mg/ml	10 µl of mg/ml	1 mg/ml
Incubation	12 h 37°C				12 h 37°C	12-16 h 37°C
Interpretation: presence of mutation	zone of inhibition: 14 mm crystal violet 13 mm deoxycholate				14 mm zone	zone

(Continued)

TABLE 4. (Continued)

Specifications	Laboratories					
	Genetic Bioassay Branch	Genex	IITRI	Litron	Meloy	Monsanto
Media for test	nutrient agar	nutrient plate	nutrient agar plate		Mueller-Hinton or nutrient agar	
Salmonella test culture	overnight broth		broth culture			
Quantity of *Salmonella*	0.1 ml of 10^8 concentration	loop full	10^8			0.1 ml
Method of *Salmonella* addition	overlay	streak	overlay		swab-streak	overlay
Compound for sensitivity test	crystal violet	crystal violet	crystal violet	deoxycholate	crystal violet	crystal violet
Method of application for compound	disc	disc	disc		disc	disc
Compound concentration	10 µl of 1 mg/ml	10 µl of 2% solution	10 µl of 1 mg/ml		100 µl of 1 mg/ml	10 µl of 1 mg/ml
Incubation	12-16 h 37°C	overnight 37°C	12 h			24-48 h 37°C
Interpretation: presence of mutation	zone	zone	zone			zone

(Continued)

TABLE 4. (Continued)

Specifications	Laboratories					
	NEIC-EPA	Northrop	Raltech	RTI	Southwest	Unilab
Media for test	minimal agar	nutrient agar	nutrient agar plates	base layer plates	nutrient agar plates	minimal glucose plates
Salmonella test culture	broth culture	nutrient broth culture		broth culture	broth culture	broth culture
Quantity of *Salmonella*	0.1 ml	0.1 ml		0.1 ml	0.1 ml	0.1 ml of 10^8 concentration
Method of *Salmonella* addition	overlay	overlay	spread on plate	overlay	overlay	overlay with His/Bio
Compound for sensitivity test	crystal violet	crystal violet	crystal violet	crystal violet	crystal violet	crystal violet
Method of application for compound	disc	disc	disc	disc	disc	disc
Compound concentration	10% solution	0.1 ml of 1 mg/ml	10 µl of 1 mg/ml		10 µl of 1 mg/ml	0.1 ml of 1 mg/ml
Incubation	24 h 37°C	24 h 37°C	overnight 37°C		overnight	overnight
Interpretation: presence of mutation	zone	zone	zone	zone	14 mm zone	zone

[a]The following laboratories do not list a procedure: Battelle, Case Western, Jefferson, Arthur D. Little, Litton, Microbiological Assoc.
[b]The following laboratories say they check for this mutation but give no instructions: EG&G Mason, Hazleton, Hilltop, SRI.

TABLE 5. AMPICILLIN RESISTANCE TEST FOR BACTERIAL STRAINS

Specifications	Reference papers			Laboratories[a,b,d]		
	Ames	de Serres	Ames revised	Bioassay Systems	Biolabs	Biotechnics
Media for test	nutrient agar plate			Mueller-Hinton plate	nutrient agar plate	
Ampicillin concentration	8 mg/ml in 0.02 N NaOH				8 mg/ml in 0.02 N NaOH	8 mg/ml in 0.2 N NaOH
Ampicillin application	streak solution on plate	disc	no data available	disc		streak on plate
Ampicillin quantity	10 µl	10 µg			10 µl	10 µl
Method of *Salmonella* addition	cross-streak	overlay		swab-streak	cross-streak	cross-streak
Incubation	12-24 h 37°C				12-24 h 37°C	16-24 h 37°C
Interpretation: presence of mutation	no zone of inhibition				no zone	no zone

(Continued)

TABLE 5. (Continued)

Specifications	Laboratories				
	Genetic Bioassay Branch	IITRI	Monsanto[c]	Meloy	NEIC-EPA
Media for test	nutrient agar plate	nutrient agar plate		Mueller-Hinton or nutrient agar	minimal agar
Ampicillin concentration		8 mg/ml in 0.02 N NaOH			
Ampicillin application	disc	streak	disc	disc	disc
Ampicillin quantity	10 µg	10 µl	10 µg		10 µg
Method of *Salmonella* addition	20 µl in overlay	cross-streak	0.1 ml overlay	swab-streak	overlay
Incubation			24-48 h 37°C	24-48 h	24 h 37°C
Interpretation: presence of mutation	no zone	no growth around streak	no zone	no zone	no zone

(Continued)

TABLE 5. (Continued)

Specifications	Laboratories			
	Northrop	Raltech	RTI[e]	Southwest
Media for test	nutrient agar	nutrient agar	base layer plates	nutrient agar
Ampicillin concentration	8 mg/ml in 0.02 N NaOH	8 mg/ml solution	8 mg/ml in 0.02 N NaOH	8 mg/ml in 0.02 N NaOH
Ampicillin application	disc	spread on plate diffuse overnight	streak	spread on plate
Ampicillin quantity	50 µl	0.1 ml	0.1 ml	0.1 ml
Method of Salmonella addition	0.1 ml in overlay	0.05 ml of 10^{-5} concentration in overlay	overlay	streak
Incubation	24 h 37°C	overnight 37°C		overnight 37°C
Interpretation: presence of mutation	no zone	colonies on plates with ampicillin should be same number as plate without ampicillin	no zone	growth

[a]The following laboratories do not list a procedure for this test: Battelle, Case Western, Jefferson, Arthur D. Little, Litton, Microbiological Assoc.
[b]The following laboratories perform this test but give no instructions: EG&G Mason, Genex, Hazleton, Hilltop, Litron, SRI.
[c]Monsanto lists an alternative procedure for Ampicillin Resistance; for details see p. 6 of their protocol.
[d]Unilab does not check for this mutation.
[e]RTI lists an alternative procedure; for details see p. 34 of their protocol.

TABLE 6. DETERMINATION OF uvrB DELETION MUTATION IN BACTERIAL STRAINS

Specifications	Reference papers			Laboratories[a,b,d]		
	Ames	de Serres	Ames revised	Bioassay Systems	Biolabs	Biotechnics
Media for test	nutrient broth agar			Mueller-Hinton plate	nutrient agar	nutrient broth plate
Salmonella culture	streak			streak	cross-streak	cross-streak
Sensitivity test	UV irradiation	no data available	no data available	UV irradiation	UV irradiation	UV irradiation
Application of test	irradiate 1/2 plate			irradiate 1/2 plate	irradiate 1/2 streak	irradiate 1/2 streak
Time period	6 sec (8 sec 98, 100)			6 sec (8 sec 98, 100)	6 sec (8 sec 98, 100)	6-8 sec
Test apparatus	G.E. 15 W germicidal lamp			G.E. 15 W germidical lamp	G.E. 15 W germidical lamp	G.E. 15 W germicidal lamp
Distance specifications	plate 33 cm from lamp			33 cm	33 cm	33 cm
Incubation	12-24 h 37°C			12-24 h 37°C	12-24 h 37°C	16-24 h 37°C
Interpretation: presence of mutation[c]	no growth on irradiated side			no growth	no growth	no growth

(Continued)

TABLE 6. (Continued)

Specifications	Laboratories				
	Genetic Bioassay Branch	IITRI	Monsanto	Meloy	NEIC-EPA
Media for test	nutrient agar plate	nutrient agar	nutrient agar	nutrient agar	nutrient agar
Salmonella culture	0.1 ml spread on plate	streak	cross-streak	streak	cross-streak
Sensitivity test	UV irradiation	UV irradiation	UV irradiation	UV irradiation	UV irradiation
Application of test	irradiate 1/2 plate	irradiate 1/2 streak	irradiate 1/2 plate	irradiate 1/2 plate	irradiate 1/2 plate
Time period	10 sec 20 sec (98, 100)	6-8 sec	6-8 sec	6 or 8 sec	8 sec
Test apparatus	15 W lamp	Sylvania G15T8 germicidal lamp	G.E. Sterilamp G36T6L	UV light	15 W germicidal lamp
Distance specifications	54 cm	33 cm	65-70 cm	33 cm	33 cm
Incubation	18-24 h 37°C	24 h 37°C	12-24 h 37°C		18-24 h 37°C
Interpretation: presence of mutation	no growth	no growth		no growth	no growth

(Continued)

TABLE 6. (Continued)

Specifications	Laboratories			
	Northrop	RTI	Southwest	Unilab
Media for test	nutrient agar	base layer plates	nutrient agar plate	Oxoid nutrient agar
Salmonella culture	0.1 ml culture in overlay	0.1 ml culture in overlay	streak	streak
Sensitivity test	UV irradiation	UV irradiation	UV irradiation	UV irradiation
Application of test	irradiate 1/2 plate	irradiate plate with "T" screen[e]	irradiate plate	irradiate sections at intervals
Time period	25 sec	6 or 8 sec	8 sec	10 sec to 1 min intervals at 10 sec intervals
Test apparatus	G.E. G64T6 lamp		UV lamp	
Distance specifications	66 cm		33 cm	30 cm
Incubation	24 h 37°C		overnight 37°C	48 h
Interpretation: presence of mutation	no growth	no growth	no growth on plate	no growth after 10 sec exposure

[a]The following laboratories do not list a procedure for this test: Battelle, Case Western, EG&G Mason, Jefferson, Litron, Arthur D. Little, Litton, Microbiological Assoc.
[b]The following laboratories say that they check for this mutation but do not give a procedure: Genex, Hazleton, Hilltop, SRI.
[c]Comment is applicable only for irradiated side of plate.
[d]Raltech says this procedure is done identically to the Ames published 1975 protocol.
[e]For details check their protocol.

SECTION 4

METABOLIC ACTIVATION SYSTEM

PREPARATION OF S-9 FRACTION: I. INDUCTION OF ANIMAL

Ames used the PCB mixture Aroclor 1254 for inducing male rats weighing approximately 200 g. He suggested using the Spraque-Dawley/BioI strain supplied from Horton Animal Laboratory. The method of induction is a single intraperitoneal injection five days before sacrificing the animal. The dosage is 500 mg of Aroclor per kg of body weight, and the mixture's concentration is 200 mg/ml suspended in corn oil. Food and water are given ad libitrium until 12 h before sacrifice, at which time the food is removed. Ames recommended Purina Laboratory Chow. The animals are sacrificed by a blow to the head followed by decapitation.

Three laboratories do not describe their induction method in their protocol as shown in Table 7. Five laboratories restrict their discussion to the strain of rat and/or the induction chemical used. Nine others use essentially the same procedure described by Ames. One laboratory differs from Ames only in the strain of rat used. Five laboratories differ from Ames in the time rats are deprived of food--24 h rather than 12. One laboratory sacrifices the rats by gassing with CO_2. One laboratory uses Aroclor 1242 rather 1254 as an alternative to corn oil; sesame oil is mentioned by one laboratory.

PREPARATION OF S-9 FRACTION: II. PREPARATION OF FRACTION

Ames gave a detailed procedure for the preparation of the fraction after sacrifice of the rat. All work is done between 0 and 4°C using sterile materials and solutions. The liver, weighing approximately 10-15 g, is removed and placed in a preweighed beaker. It is washed twice with cold 0.15 M KCl. The first wash is with 1 ml KCl solution per gram wet liver, and the second with 3 ml KCl solution per gram wet liver. The liver is then minced with sterile scissors and homogenized using a Potter-Elvehjen homogenizer with a Teflon pestle. After homogenizing, a Sorvall RC_2-B centrifuge with a SS-34 head is used for 10 min at 8700 rev/min (9000xg). The supernatant is decanted and saved. The decanted supernatant (fraction) is stored in a 2 ml plastic liquid nitrogen storage tubes. It is quick-frozen on dry ice and stored in a Revco freezer at -80°C.

Five laboratories make no mention of the procedure as shown in Table 8. Five others list only the centrifuge speed or organ used or storage temperature. Eight laboratories give essentially the same procedure as the one described by Ames. Three laboratories differ only in the amount of fraction

in a storage container. One expands the Ames protocol to include a detailed homogenizing procedure. One laboratory used a Polytron J21C centrifuge instead of a Sorvall. Two use a sucrose wash in combination with two KCl washes. One laboratory that uses a sucrose wash also centrifuges for 25 min rather than 10.

STORAGE OF S-9 FRACTION

Ames recommended that the S-9 fraction be stored in liquid nitrogen plastic storage tubes. He places 2 ml in a container, quick freezes on dry ice, and stores in a Revco freezer at -80°C. The only variations from this procedure are in the types of freezing containers and the amounts in the containers.

The majority of laboratories store in 2 ml quantities as shown in Table 9. Seven laboratories do not list a procedure, and seven others give only the temperature at which the fraction is stored. Eleven laboratories describe the quantities stored and/or the containers used.

PREPARATION OF S-9 MIX

Ames listed the following ingredients and amounts for the S-9 mix:

S-9	-	0.04-0.1 ml/ml mix
$MgCl_2$	-	8 µmole/ml mix
KCl	-	33 µmole/ml mix
G-6-P	-	5 µmole/ml mix
NADP	-	4 µmole/ml mix
sodium phosphate buffer	-	100 µmole/ml mix

He recommended keeping the mix on ice for the assay procedure and only retaining it for several hours.

Most laboratories use the quantities listed above as described in Table 10. One laboratory does not list the quantities in their protocol. The only significant difference in the protocols is that one laboratory listed the use of 100 µmole G-6-P/ml mix, 5 µmole NADP/ml mix, and 4 µmole sodium phosphate buffer/ml mix. One laboratory omits KCl as a component in the mix. These differences may be typographical errors.

TABLE 7. PREPARATION OF S-9 FRACTION: I-INDUCTION OF ANIMAL

Specifications	Reference papers			Laboratories[a,b,c]		
	Ames	de Serres	Ames revised	Battelle	Biolabs	Biotechnics
Induction chemical	Aroclor 1254	Aroclor 1254		Aroclor 1254	Aroclor 1242	Aroclar 1254
Animal type	male rat			male rat	male rat	male rat
Animal weight	~200 g			~200 g	200-300 g	
Animal strain	Spraque-Dawley/Bio I		no data available		Charles River-Wistar	
Supplier	Horton Animal Lab				Charles River Breeding Lab	
Induction method	single i.p. injection			single i.p. injection	single i.p. injection	injection
Inducer dosage	500 mg/kg			0.5 mg/g	500 mg/kg	500 mg/kg
Inducer concentration	200 mg/ml			200 mg/ml	200 mg/ml	200 mg/ml
Inducer solvent	corn oil			corn oil	corn oil	corn oil, sesame oil
Food and H_2O intake	ad libitum				ad libitum	H_2O, ad libitum food, normal
Food variety	Purina Lab Chow				Purina Lab Chow	Purina Lab Chow
Food removed	12 h before sacrifice			24 h before sacrifice	12 h before sacrifice	12 h before sacrifice
Sacrifice day	5th day after injection			5th day after injection	5th day after injection	5th day after injection
Sacrifice technique	blow to head and decapitate			blow to head and decapitate	cervical separation decapitate and bleed	

(Continued)

TABLE 7. (Continued)

Specifications	Laboratories					
	Case Western	EG&G Mason	Genetic Bioassay Branch	Genex	Hazleton	Hilltop
Induction chemical		Aroclor 1254	Aroclor 1254	Aroclor 1254	Aroclor 1254	Aroclor 1254
Animal type	hamster, rat	male rat	rat	adult male rat	male rat	male rat
Animal weight			~200 g	200-250 g		200 g
Animal strain		Spraque-Dawley	Charles River CDI	Fisher 344	Spraque-Dawley	Spraque-Dawley
Supplier						
Induction method		single i.p. injection	i.p. injection	single i.p. injection	single i.p. injection	single i.p. injection
Inducer dosage		500 mg/kg	500 mg/kg	500 mg/kg	500 mg/kg	500 mg/kg
Inducer concentration		200 mg/ml	200 mg/ml		200 mg/ml	200 mg/ml
Inducer solvent		corn oil	corn oil	corn oil	corn oil	corn oil
Food and H_2O intake			H_2O, ad libitum			ad libitum
Food variety						
Food removed		12 h before sacrifice	one day before sacrifice	24 h before sacrifice	prior to sacrifice	12 h before sacrifice
Sacrifice day		5th day after injection	5th day after injection	5th day after injection	5th day after injection	5th day after injection
Sacrifice technique		decapitate	cervical dislocation	guillotine	decapitate	stun decapitate

(Continued)

TABLE 7. (Continued)

Specifications	Laboratories						
	IITRI	Jefferson	Litron	Arthur D. Little	Litton	Meloy	Monsanto
Induction chemical	Aroclor 1254	Aroclor 1254	Aroclor 1254	Aroclor 1254	Aroclor 1254	Aroclor 1254	Aroclor 1254
Animal type	male rat	male rat	adult rat	male rat	adult male rat		male rat
Animal weight	180-200 g	300 g		200 g			200 g
Animal strain	Spraque-Dawley	Spraque-Dawley	Spraque-Dawley	Spraque-Dawley	Spraque-Dawley		Spraque-Dawley
Supplier							
Induction method	single i.p. injection	single i.p. injection		single i.p. injection			i.p. injection
Inducer dosage	500 mg/kg	500 mg/kg	500 mg/kg	0.5 mg/g		0.5 cm^3	500 mg/kg
Inducer concentration	200 mg/ml	200 mg/ml		200 mg/ml		200 mg/ml	200 mg/ml
Inducer solvent	corn oil	corn oil		corn oil		corn oil	corn oil
Food and H_2O intake		ad libitum					
Food variety							Purina Lab Chow
Food removed		24 h before sacrifice		12 h before sacrifice			12 h before sacrifice
Sacrifice day	5th day after injection	5th day after injection	5th day after treatment	5th day after injection			5th day after injection
Sacrifice technique		gas with CO_2		blow to head decapitate			

(Continued)

TABLE 7. (Continued)

Specification	Laboratories					
	NEIC-EPA	Northrop	Raltech	RTI	SRI	Southwest
Induction chemical	Aroclor 1254	Aroclor 1254	Aroclor 1254	Aroclor 1254	Aroclor 1254	Aroclor 1254
Animal type	rat	young male rat	rat	male rat	adult male rat	male rat
Animal weight		100-150 g		200 ± 20 g	180-250 g	~200 g
Animal strain		Charles River CD-I		Charles River strain SD	Fischer 344	Spraque-Dawley
Supplier						
Induction method				i.p. injection	single i.p. injection	single i.p. injection
Inducer dosage				500 mg/kg	500 mg/kg	500 mg/kg
Inducer concentration				200 mg/ml		200 mg/ml
Inducer solvent				corn oil (Mazola)		corn oil
Food and H_2O intake				H_2O, ad libitum		
Food variety						
Food removed				12 h before sacrifice	1 day before sacrifice	12 h before sacrifice
Sacrifice day	5th day after injection			5th day after injection	5th day after injection	5th day after injection
Sacrifice technique				guillotine		blow to head decapitate

[a]The following laboratories do not list a procedure for induction: Bioassay Systems, Microbiological Assoc., Unilab.
[b]Some laboratories may have a separate protocol for induction that was not submitted.
[c]Spelling and symbolic notation are as shown in the original protocols.

TABLE 8. PREPARATION OF S-9 FRACTION: II-PREPARATION OF FRACTION

Specifications	Reference papers			Laboratories[a]		
	Ames	de Serres	Ames revised	Battelle	Biolabs	Biotechnics
Temperature requirement	0-4°C				0-4°C	0-4°C
Organ used	liver			liver	liver	liver
Weight of organ	10-15 g					10-15 g
Container for organ during procedure	sterile preweighed beaker			cold preweighed beaker	cold sterile beaker	preweighed beaker
Wash solution	0.15 M KCl			0.5 M KCl	0.15 M KCl	0.15 M KCl
Number of washes	2			2	2	2
Concentration of washes[b]	1-1 ml 2-3 ml			1-10 ml KCl/liver 2-3 ml	1-1 ml 2-3 ml	1-1 ml 2-3 ml
Mincing apparatus	sterile scissors		no data available	sterile scissors	sterile scissors	sterile scissors
Homogenizer type[d]	Potter-Elvehjen with Teflon pestle			low speed motor driven pestle	Potter-Elvejhem with Teflon pestle	Potter-Elvehjem with Teflon pestle
Homogenizing technique				pestle passed through 4x		
Centrifuge temperature				4°C		
Centrifuge type	Sorvall RC_2-B					Sorvall RC_2-B
Centrifuge time	10 min			10 min	10 min	10 min
Centrifuge speed	8700 rpm (9000xg)			9000g	9000g (8700 rpm)	9000g (8700 rpm)
Centrifuge head	SS-34					SS-34
Storage container	2 ml plastic liquid nitrogen storage tubes			small culture tubes		small sterile plastic tubes
Amount in container	2 ml			3 ml	2 ml	2 ml
Method of freezing	dry ice			dry ice	quick freeze	dry ice
Storage place, temperature	Revco frezer -80°C	-70°C or below		Revco -80°C	-80°C	Revco -80°C

(Continued)

TABLE 8. (Continued)

Specifications	Laboratories					
	EG&G Mason	Genetic Bioassay Branch	Genex	Nazleton	Hilltop	IITRI
Temperature requirement	0-4°C	ice bath	ice bath		0-4°C	
Organ used	liver	liver	liver	liver	liver	
Weight of organ						
Container for organ during procedure	preweighed sterile beaker	preweighed beaker	preweighed sterile beaker	sterile ice cold beaker	preweighed sterile beaker	
Wash solution	0.15 M KCl	1.15% KCl 0.2 M sucrose	0.15 M KCl	0.15 M KCl	0.15 M KCl	
Number of washes	2	several KCl 2 sucrose washes	2	2	2	
Concentration of washes[b]	1-20 ml/liver 2-3 ml	sucrose 2:1 ratio	1-1 ml 2-3 ml	1-1 ml 2-3 ml	1-KCl 2-3 ml	
Mincing apparatus	sterile scissors		sterile scissors	sterile scissors	sterile scissors	
Homogenizer type[d]	Potter-Elvehjen with Teflon pestle	50 ml Potter-Elvehjen	Potter-Elvehjem		Potter-Elvehjem Teflon pestle	
Homogenizing technique		1 down & up stroke				
Centrifuge temperature		4°C				
Centrifuge type	Sorvall SS-3			refrigerated		
Centrifuge time	10 min	25 min	10 min		10 min	
Centrifuge speed[d]	9000xg	9000xg	9000xg	9000xg	9000xg	9000xg
Centrifuge head	SS-34					
Storage container	freezing ampules	2 ml polypropylene freezing vials				
Amount in container	small aliquots	2 ml				
Method of freezing	liquid nitrogen	liquid nitrogen	dry ice	dry ice	quick freeze	
Storage place, temperature		Revco -70°C	-80°C	freezer	-80°C	-70°C

(Continued)

TABLE 8. (Continued)

Specifications	Laboratories					
	Jefferson	Litron	Arthur D. Little	Monsanto	Meloy	NEIC-EPA
Temperature requirement	0-5°C		4°C		4°C	
Organ used	liver		liver		liver	liver
Weight of organ			10-15 g			
Container for organ during procedure	preweighed sterile petri plate		sterile preweighed beaker	sterile beaker	preweighed sterile tripour beaker	
Wash solution	0.15 M KCl		0.15 M KCl	KCl	0.15 M KCl	
Number of washes	2		2	2	2	
Concentration of washes[b]	1... 2-3 ml		1-1 ml 2-3 ml	1-1 ml (0.5 M KCl) 2-3 ml (0.15 M KCl)	1-5 ml/liver 2-3 ml	
Mincing apparatus	sterile scissors		sterile scissors		sterile scissors	
Homogenizer type[d]	Potter-Elvehjan with Teflon pestle		low speed motor driven pestle			
Homogenizing technique			pestle passed through liver 3x			
Centrifuge temperature			4°C			
Centrifuge type				refrigerated	Polytron J21C	
Centrifuge time	15 min		10 min	10 min	10 min	
Centrifuge speed[d]	9000xg	9000g	9000g	9000xg	8500 rpm	9000xg
Centrifuge head					JA-14	
Storage container			small storage tubes		sterile dram vials	2 ml plastic vials
Amount in container			2 ml	2 or 5 ml	2 ml	
Method of freezing	quick freeze			dry ice		dry ice
Storage place, temperature	-70°C		freezer -80°C	-70 to -80°C	Revco	-80°C

(Continued)

TABLE 8. (Continued)

Specifications	Laboratories				
	Northrop	Raltech	RTI[c]	SRI	Southwest
Temperature requirement				ice bath	
Organ used		liver	liver	liver	liver
Weight of organ					
Container for organ during procedure			sterile jar	preweighed sterile beaker	tared beaker
Wash solution			0.25 M sucrose	0.15 M KCl	0.15 M KCl
Number of washes			2	2	2
Concentration of washes[b]				1-1 ml 2-3 ml	1-1 ml 2-3 ml
Mincing apparatus				sterile surgical scissors	
Homogenizer type[d]			Potter-Elvehjem with Teflon pestle	Potter-Elvehjem	Potter-Elvejhem with Teflon pestle
Homogenizing technique			4 strokes		
Centrifuge temperature					4°C
Centrifuge type			Sorvall		Sorvall RC refrigerated
Centrifuge time			10 min	10 min	10 min
Centrifuge speed			9000g (10,000 rpm)	9000xg	9000g
Centrifuge head			SS-34		
Storage container			cryotubes		sterile capped plastic test tubes
Amount in container			2-5 ml		3 ml, 10 ml
Method of freezing			quick freeze	dry ice	dry ice
Storage place, temperature	Revco -70°C		-80°C 2 months	-80°C	Revco -80°C

[a]The following laboratories do not give instructions for the procedure: Bioassay Systems, Case Western, Litton, Microbiological Assoc., Unilab.
[b]Wash concentrations will be given in ml KCl/g wet liver unless otherwise noted.
[c]RTI uses sucrose washes; for further information see their protocol.
[d]Spelling and symbolic notation are as shown in the original protocols.

TABLE 9. STORAGE OF S-9 FRACTION

Specifications	Reference papers			Laboratories[a]		
	Ames	de Serres	Ames revised	Battelle	Biolabs	Biotechnics
Storage container	2 ml liquid nitrogen plastic storage tubes			small culture tubes		freezing ampules
Amount in container	2 ml			3 ml	2 ml	small aliquot
Method of freezing	freeze on dry ice		no data available	dry ice	quick freeze	liquid nitrogen
Length of storage						
Storage place, temperature	Revco freezer -80°C	-70°C		Revco freezer -80°C	-80°C	

(Continued)

TABLE 9. (Continued)

Specifications	Laboratories							
	EG&G Mason	Genetic Bioassay Branch	Genex	Hazleton	Hilltop	IITRI	Jefferson	Arthur D. Little
Storage container	freezing ampules	2 ml polypropylene freezing vials						small storage tubes
Amount in container	small aliquot	2 ml						2 ml
Method of freezing	liquid nitrogen	liquid nitrogen	dry ice	dry ice	quick freeze		quick freeze	dry ice
Length of storage								
Storage place, temperature		Revco -70°C	-80°C	freezer	-80°C	-70°C	-70°C	freezer -80°C

(Continued)

TABLE 9. (Continued)

Specifications	Laboratories						
	Monsanto	Meloy	NEIC-EPA	Northrop	RTI	SRI	Southwest
Storage container		sterile dram vials	2 ml plastic vials		cryotubes		sterile capped plastic test tubes
Amount in container	2 ml or 5 ml	2 ml			2-5 ml		3 ml, 10 ml
Method of freezing	dry ice		dry ice		quick freeze	dry ice	dry ice
Length of storage					2 months		
Storage place, temperature	-70 to -80°C	Revco	-80°C	Revco -70°C	-80°C	-80°C	Revco -80°C

[a]The following laboratories do not list storage information: Bioassay Systems, Case Western, Litron, Litton, Microbiological Assoc., Raltech, Unilab.

TABLE 10. PREPARATION OF S-9 MIX

Specifications[b]	Reference papers			Laboratories[a,P]			
	Ames	de Serres	Ames revised	Battelle	Bioassay Systems	Biolabs	Biotechnics
S-9 quantity	0.04-0.1 ml			0.1 ml	2-5 ml/50 ml	50 µl	0.04-0.1 ml
$MgCl_2$ quantity	8 µmole			8 µmole	1 ml/50 ml	8 µmole	8 µmole
KCl quantity	33 µmole	no data available	no data available	33 µmole		33 µmole	33 µmole
G-6-P quantity	5 µmole			5 µmole	0.25 ml/50 ml	5 µmole	5 µmole
NADP quantity	4 µmole			4 µmole	2 ml/50 ml	4 µmole	4 µmole
Sodium phosphate buffer quantity	100 µmole			100 µmole	25 ml/50 ml	100 µmole	100 µmole
Storage	several hours on ice				several hours on ice	filter on ice	several hours on ice

(Continued)

TABLE 10. (Continued)

Specifications	Laboratories							
	EG&G Mason[c]	Genetic Bioassay Branch	Genex[d]	Hazleton[e]	Hilltop[f]	IITRI[g]	Jefferson	Litron[h]
S-9 quantity	0.1 ml	0.05-0.1 ml	1 ml/10 ml	0.05 ml		0.1 ml	0.3 ml	1 ml/10 ml
$MgCl_2$ quantity	0.02 ml	0.02 ml	16.2 mg/10 ml	0.02 ml	8 µmole	8 µmole	8 µmole	0.2 ml/10 ml
KCl quantity	0.02 ml		24.6 mg/10 ml	0.02 ml	33 µmole	33 µmole	33 µmole	0.2 ml/10 ml
G-6-P quantity	0.1 ml	1.41 mg	14.0 mg/10 ml	0.05 ml	100 µmole	5 µmole	5 µmole	0.05 ml/10 ml
NADP quantity	0.1 ml	3.06 mg (filtered)	31.5 mg/10 ml	0.08 ml	5 µmole	4 µmole	4 µmole	0.4 ml/10 ml
Sodium phosphate buffer quantity	0.1 ml	0.5 ml	5 ml/10 ml	0.5 ml	4 µmole	100 µmole	100 µmole	5 ml/10 ml
Storage				1 day on ice		sterile filter -20°C	ice bath	

(Continued)

TABLE 10. (Continued)

Specifications	Laboratories					
	Arthur D. Little	Litton[h]	Monsanto	Microbiological Assoc.[i]	Meloy[j]	NEIC-EPA
S-9 quantity	0.04-0.1 ml	100 µl	0.04-0.1 ml	100 µl	1.25-12.5 ml	2 ml/50 ml
$MgCl_2$ quantity	8 µmole	8 µmole	8 µmole	8 mM		
KCl quantity		33 µmole	33 µmole	33 mM	0.267 ml/12.5 ml	1 ml/50 ml
G-6-P quantity	5 µmole	5 µmole	5 µmole	5 mM	0.067 ml/12.5 ml	0.25 ml/50 ml
NADP quantity	4 µmole	4 µmole	4 µmole	4 mM	0.533 ml/12.5 ml	2 ml/50 ml
Sodium phosphate buffer quantity	100 µmole	100 µmole	100 µmole	0.1 M	6.667 ml/12.5 ml	25 ml/50 ml
Storage	on ice		filter sterilize on ice several hours	filter on ice		

(Continued)

TABLE 10. (Continued)

Specifications	Laboratories				
	Northrop	Raltech[k]	SRI[l]	Southwest[m]	Unilab[o]
S-9 quantity	0.1 ml	0.4 ml/9.6 ml	1 ml/10 ml		0.04-0.1 ml
$MgCl_2$ quantity	8 µmole	0.2 ml/9.6 ml	0.2 ml/10 ml	1.62 mg	8 µmole
KCl quantity	33 µmole			2.457 mg	33 µmole
G-6-P quantity	5 µmole	0.5 ml/9.6 ml	0.05 ml/10 ml	1.413 mg	5 µmole
NADP quantity	4 µmole	0.4 ml/9.6 ml	0.4 ml/10 ml	3.15 mg	4 µmole
Sodium phosphate buffer quantity	100 µmole	5 ml/9.6 ml	5 ml/10 ml	n	100 µmole
Storage		filter on ice 1 day		filter on ice	on ice

[a]The following laboratories do not give instructions for preparation: Case Western.
[b]Quantities are be given per ml of mix unless noted otherwise.
[c]An aliquot of 0.56 ml H_2O is added per ml mix.
[d]An aliquot of 3.35 ml H_2O is added per 10 ml mix.
[e]An aliquot of 0.28 ml H_2O is added per ml mix; all ingredients except NADP and G-6-P are filter sterilized.
[f]G-6-P, NADP, and buffer quantities may be typographical errors.
[g]All ingredients except S-9 are mixed prior to use and stored at -20°C.
[h]An aliquot of 3.15 ml H_2O is added per 10 ml mix.
[i]Numbers given are concentrations of ingredients.
[j]An aliquot of 3.1 ml H_2O is added per 9.6 ml mix.
[k]An aliquot of 3.5 ml H_2O is added per 9.6 ml mix.
[l]An aliquot of 3.35 ml H_2O is added per 10 ml mix.
[m]The mix is made up in 100 ml quantities and stored in 9 ml aliquots without S-9. It is stored at -80°C in sterile tissue culture screw cap vials.
[n]Buffer containers 11.502 mg/ml dibasic sodium phosphate and 2.502 mg/ml monobasic sodium phosphate.
[o]Mutazyme is purchased from Meloy.
[p]RTI uses an NADPH generating system; for further information, see their protocol.

SECTION 5

STOCK SOLUTIONS

SODIUM PHOSPHATE BUFFER

Ames recommended using a 0.2 M sodium phosphate buffer in the S-9 mix. The buffer's pH is 7.4 and the amount used per ml of mix is 100 µmole. One laboratory in Table 11 does not say what buffer they use. Three laboratories list a storage procedure with two of them also listing the pH. Five laboratories list the pH and amount of buffer used. Two others list the pH and the molarity as described by Ames. Eight laboratories give essentially the same description as Ames, and four use different buffer molarities (0.125, 0.4, 0.1 or 1 M). The laboratory using 1 M buffer uses 0.1 ml buffer/ml mix. One other uses an NADPH generating system. One laboratory uses a mixture of monobasic and dibasic sodium phosphate.

Storage information for the buffer is indicated by only eight laboratories Seven of the laboratories store the buffer at refrigerator temperatures. One laboratory stores the buffer at room temperature.

MICROSOMAL SALT SOLUTION

Ames said that the microsomal salt solution should consist of 0.4 M $MgCl_2$ and 1.65 M KCl. The quantities per ml of mix are 8 µmole $MgCl_2$ and 33 µmole KCl.

Twenty laboratories shown in Table 12 list the same quantities and/or molarity as Ames. One laboratory does not give any information for the microsomal salt solution. One laboratory gives the molarity as 0.4 M for both $MgCl_2$ and KCl. One laboratory reports 0.65 M concentration for KCl. Another reports standard molarities but gives gram weights 10x higher than expected. One laboratory uses an NADPH generating system; details on their solutions may be found in their protocol.

Storage information usually consists of refrigeration after autoclaving. One laboratory autoclaves and stores the solution at room temperature.

GLUCOSE STOCK SOLUTION

Ames listed no directions for the preparation of a glucose stock solution. Five of the laboratories shown in Table 13 make no mention of a glucose stock solution. Sixteen laboratories indicate the use of a 2% solution. One

laboratory uses a 0.8% solution and two use a 1% solution. One uses a stock solution of 20% but does not give the quantity of this solution that is used in the agar.

HISTIDINE/BIOTIN STOCK SOLUTION

Ames reported a molarity of 0.5 mM for both the histidine and the biotin in the His/Bio stock solution or quantities of 8.7136 mg/l histidine and 11.1045 g/l biotin. He recommended using 10 ml of the solution per 100 ml top agar.

Fourteen of the laboratories shown in Table 14 use these amounts. Two do not give instructions for this solution. One laboratory reports using trace amounts of His/Bio. Four use 0.05 mM solutions. One laboratory uses 39 mg/l of histidine. One laboratory uses 10 µg/ml of histidine and 12 µg/ml of biotin in their stock solution. One laboratory uses 0.0976 g biotin/l and 0.0525 g histidine/l in the solution. Another uses 61.0 mg biotin/500 ml and 48.0 mg histidine/500 ml and 100 ml of the stock solution per liter of top agar.

VBME SALT SOLUTION

Ames did not give instructions for the preparation of the VBME solution.

Vogel's reference gives the following gram amounts per liter of solution:

$MgSO_4 \cdot 7\ H_2O$	- 10 g
Citric Acid $\cdot\ H_2O$	- 100 g
K_2HPO_4 · Anhydrous	- 500 g
$NaNH_4 \cdot HPO_4 \cdot 4\ H_2O$	- 175 g
H_2O	- 670 ml

The pH is 7.0 and the solution is a 50x concentration. Fourteen of the laboratories in Table 15 use these same instructions and 10 give none. One laboratory gives the same gram amounts but lists the concentration of the solutions at 25x.

TABLE 11. SODIUM PHOSPHATE BUFFER PREPARATION

Specifications	Reference papers			Laboratories[a,j,i]			
	Ames	de Serres	Ames revised	Battelle	Bioassay[c] Systems	Biolabs	Biotechnics
Concentration	0.2 M				0.2 M		0.2 M
pH	7.4	no data available	no data available	7.4	7.4	7.4	7.4
Quantity used[b]	100 µmole/ml mix			100 µmole		100 µmole	
Storage	autoclave refrigerate				autoclave refrigerate		autoclave refrigerate

(Continued)

TABLE 11. (Continued)

Specifications	Laboratories								
	EG&G Mason[d]	Genetic Bioassay Branch	Genex	Hazleton	Hilltop	IITRI[f]	Jefferson	Litron	Arthur D. Little
Concentration	1 M	0.2 M	0.2 M	0.2 M		0.4 M		0.2 M	0.2 M
pH	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4
Quantity used[b]	0.1 ml	0.5 ml	0.5 ml	0.5 ml	100 µmole	100 µmole	100 mM	0.5 ml	100 µmole
Storage		autoclave				autoclave refrigerate			autoclave refrigerate

(Continued)

TABLE 11. (Continued)

Specifications	Laboratories								
	Litton	Monsanto	Microbiological Assoc.	Meloy[g]	NEIC-EPA[h]	Northrop	Raltech	SRI	Unilab
Concentration	0.2 M		0.1 M				0.2 M	0.2 M	0.125 M
pH	7.4	7.4	7.4		7.4	7.4	7.4	7.4	7.4
Quantity used[b]	100 µmole					100 µmole			100 µmole
Storage	autoclave	autoclave refrigerate		autoclave room temperature	autoclave				autoclave refrigerate

[a]The following laboratory does not list specifications: Case Western.
[b]Quantity is expressed as quantity per ml S-9 mix.
[c]Concentration is 28.39 g Na_2HPO_4 per liter H_2O.
[d]EG&G Mason uses NaH_2PO_4.
[e]Genetic Bioassay Branch uses 7.76 g KH_2PO_4/l and 20.39 g Na_2HPO_4/l.
[f]IITRI uses 55.2 g KH_2PO_4/l and 107.2 g Na_2HPO_4/l.
[g]Meloy uses 2.84 g Na_2HPO_4/100 ml H_2O.
[h]NEIC-EPA uses 21.45 g Na_2HPO_4 and 4.14 g Na_2HPO_4/550 ml H_2O.
[i]Southwest uses 1.278 g dibasic sodium phosphate per liter and 0.278 g monobasic sodium phosphate per liter as buffers.
[j]RTI uses an NADPH generating system; for details on solutions, see their protocol.

TABLE 12. MICROSOMAL SALT SOLUTION PREPARATION

Specifications	Reference papers			Laboratories[a,c]			
	Ames	de Serres	Ames revised	Battelle	Bioassay Systems	Biolabs	Biotechnics
$MgCl_2$ concentration	0.4 M				0.4 M (81.32 g/100 ml)		0.4 M
Quantity used[b]	8 µmole/ml S-9 mix			8 µmole		8 µmole	
KCl concentration	1.65 M	no data available	no data available				
Quantity used	33 µmole/ml mix			33 µmole	1.65 M (12.30 g/100 ml)	33 µmole	0.65 M
Storage	autoclave refrigerate			autoclave refrigerate	autoclave refrigerate		autoclave refrigerate

(Continued)

TABLE 12. (Continued)

Specifications	Laboratories							
	EG&G Mason	Genetic Bioassay Branch	Genex	Hazleton	Hilltop	IITRI	Jefferson	Litron
$MgCl_2$ concentration	0.4 M	0.4 M (81.32 g/l)	16.2 mg/10 ml H_2O	0.4 M		32 mM (6.5 g/l)		0.4 M
Quantity used[b]	0.02 ml			0.02 ml	8 µmole	8 µmole	8 mM	0.2 ml/10 ml
KCl concentration	1.65 M	1.65 M (123.02 g/l	24.6 mg/10 ml H_2O	1.65 M		132 mM (9.84 g/l)		0.4 M
Quantity used	0.02 ml			0.02 ml	33 µmole	33 µmole	33 mM	0.2 ml/10 ml
Storage		autoclave				autoclave refrigerate		

(Continued)

TABLE 12. (Continued)

Specifications	Laboratories					
	Arthur D. Little	Litton	Monsanto	Microbiological Assoc.	Meloy	NEIC-EPA
$MgCl_2$ concentration	0.4 M	0.4 M	0.4 M		8.13 g/100 ml	0.4 M (40.66 g/500 ml)
Quantity used	8 µmole	8 µmole	8 µmole	8 µmole		
KCl concentration	1.65 M	1.65 M	1.65 M		12.39 g/100 ml	1.65 M (61.42 g/500 ml)
Quantity used		33 µmole	33 µmole	33 µmole		
Storage	autoclave refrigerate	autoclave	autoclave refrigerate		autoclave room temperature	autoclave refrigerate

(Continued)

TABLE 12. (Continued)

Specifications	Laboratories				
	Northrop	Raltech	SRI	Southwest	Unilab
$MgCl_2$ concentration		0.4 M	0.4 M		0.4 M
Quantity used	8 µmole			1.62 mg	8 µmole
KCl concentration		1.65 M	1.65 M		1.65 M
Quantity used	33 µmole			2.457 mg	33 µmole
Storage					autoclave refrigerate

[a]The following laboratory gives no instructions for preparation: Case Western.
[b]Quantity used is expressed in quantity per ml S-9 mix.
[c]RTI uses an NADPH generating system; for details on solutions, see their protocol.

TABLE 13. GLUCOSE STOCK SOLUTION

Specifications	Reference papers			Laboratories[a]					
	Ames	de Serres	Ames revised	Battelle	Bioassay Systems	Biolabs	Genetic Bioassay Branch	Genex	Hilltop
Glucose concentration	2%			2%	40%	2%	60 g/200 ml	1%	2%
Amount used		no data available	no data available		50 ml/1 agar		200 ml/2655 ml agar		
Storage					filter		autoclave		

(Continued)

TABLE 13. (Continued)

Specifications	Laboratories							
	IITRI	Jefferson	Litron	Arthur D. Little	Litton	Meloy	Monsanto	NEIC-EPA
Glucose concentration	2%	2%	2%	2%	2%	40%	20 g/l	2%
Amount used						10 ml/500 ml agar		
Storage							autoclave or filter	

(Continued)

TABLE 13. (Continued)

Specifications	Laboratories						
	NEIC-EPA	Northrop	Raltech	RTI	SRI	Southwest	Unilab
Glucose concentration	2%	20 g/l	400 g/l	20 g/l	10 g/l	200 g/l	2%
Amount used			50 ml/l agar				
Storage			filter room temperature 1 yr			autoclave	autoclave

[a]The following laboratories do not give information on glucose stock solutions: Biotechnics, Case Western, Microbiological Assoc., EG&G Mason, Hazleton.

TABLE 14. HISTIDINE/BIOTIN STOCK SOLUTION

Specifications	Reference papers			Laboratories[a]				
	Ames	de Serres	Ames revised	Battelle	Bioassay Systems	Biolabs	Biotechnics	EG&G Mason
Biotin concentration	0.5 mM			0.5 mM	5 mM	0.5 mM	0.0005 M	0.5 mM
Histidine concentration	0.5 mM	no data available	no data available	0.5 mM	5 mM	0.5 mM	0.0001 M	0.5 mM
Storage					filter room temperature		filter 4°C	
Ratio: ml used/ml agar	10/100			20/200	1/108	10/100	10/100	10/100

(Continued)

TABLE 14. (Continued)

Specifications	Laboratories					
	Genetic Bioassay Branch	Genex	Hilltop	IITRI	Jefferson	Litron
Biotin concentration	0.0976 g/l	0.5 mM	10 µg/ml agar	0.5 mM	0.5 mM	0.05 mM
Histidine concentration	0.0525 g/l	0.5 mM	12 µg/ml agar	0.5 mM	0.5 mM	0.05 mM
Storage	autoclave refrigerate 1 week			filter -20°C		
Ratio: ml used/ml agar				10/100	10/100	

(Continued)

TABLE 14. (Continued)

Specifications	Laboratories					
	Arthur D. Little	Litton	Monsanto	Microbiological Assoc.	Meloy	NEIC-EPA
Biotin concentrations	trace	0.05 mM	0.5 mM	0.5 mM	244.4 mg/200 ml	0.5 mM
Histidine concentrations	trace	0.05 mM	0.5 mM	0.5 mM	20.96 mg/200 ml	0.5 mM
Storage			filter refrigerate		filter 4°C	filter refrigerate
Ratio: ml used/ml agar			10/100	10/100	10/100	20/200

(Continued)

TABLE 14. (Continued)

Specifications	Laboratories					
	Northrop	Raltech	RTI	SRI	Southwest	Unilab
Biotin concentration		0.5 mM	61.0 mg/500 ml	0.05 mM	0.5 mM	0.05 mM
Histidine concentration	39 mg/l	0.5 mM	48.0 mg/500 ml	0.5 mM	0.5 mM	0.05 mM
Storage		filter refrigerate 1 yr	autoclave			filter
Ratio: ml used/ml agar		10/100	100/1	10/100	10/100	10/100

[a]The following laboratories do not give information on their His/Bio solutions: Case Western, Hazleton.

TABLE 15. VOGEL BONNER MEDIUM E SALT SOLUTION

Specifications	Reference papers				Laboratories[a]					
	Ames	de Serres	Ames revised	Vogel	Bioassay Systems	Genetic Bioassay Branch	Genex	Hilltop[b]	IITRI	Jefferson[b]
H_2O quantity				670 ml	670 ml	600 ml			670 ml	0.2 g
$MgSO_4 \cdot 7\ H_2O$ quantity	no data available	no data available	no data available	10 g	10 g	10 g	0.02%	0.2 g	10 g	2 g
Citric Acid $\cdot\ H_2O$ quantity				100 g	100 g	100 g	0.2%	5 g	100 g	10 g
K_2HPO_4 $\cdot$ anhydrous quantity				500 g	500 g	500 g	1%	10 g	500 g	3.5 g
$NaNH_4HPO_4 \cdot 4\ H_2O$ quantity				175 g	175 g	175 g	0.35%	3.5 g	175 g	
Final volume				1 l		1 l			1 l	
Dilution strength				50x						
pH				7.0						
Storage				autoclave		filter autoclave			autoclave refrigerate	

(Continued)

TABLE 15. (Continued)

Specifications	Laboratories								
	Monsanto[b]	Meloy	NEIC-EPA	Northrop[b]	Raltech	RTI	SRI[b]	Southwest	Unilab[b]
H_2O quantity		700 ml	670 ml		670 ml	670 ml		670 ml	
$MgSO_4 \cdot 7\ H_2O$ quantity	0.2 g	10 g	10 g	0.2 g	10 g	10 g	0.2 g	10 g	0.2 g
Citric Acid $\cdot\ H_2O$ quantity	2 g	100 g	100 g	2.0 g	100 g	100 g	2 g	100 g	2 g
K_2HPO_4 $\cdot$ anhydrous quantity	10 g	500 g	500 g	10 g	500 g	500 g	10 g	500 g	10 g
$NaNH_4HPO_4 \cdot 4\ H_2O$ quantity	3.5 g	175 g	175 g	3.5 g	175 g	175 g	3.5 g	175 g	3.5 g
Final volume		1 l	1 l					1 l	
Dilution strength	50x	25x						50x	
pH									
Storage	room temperature				autoclave room temperature 1 yr	autoclave room temperature indefinitely		autoclave	autoclave

[a]The following laboratories do not give instructions for preparation: Ames, Battelle, Biolabs, Biotechnics, Case Western, EG&G Mason, Hazleton, Litron, Arthur D. Little, Litton, Microbiological Assoc.
[b]Quantities given are added to 1 liter of agar.

SECTION 6

MEDIA

TOP AGAR OVERLAYS

Ames recommended a solution of 0.6% Difco agar and 0.5% NaCl for top agar, which should be stored in 100 ml bottles at room temperature. Before using, the top agar is melted in a steam bath and 10 ml of a His/Bio solution is added to 100 ml of top agar. The His/Bio solution consists of a 0.5 mM concentration of histidine and a 0.5 mM concentration of biotin. Aliquots of 2 ml are pipetted into culture tubes and kept warm until use.

Nine laboratories prepare overlays by this procedure as shown in Table 16. Nine others give the quantities of the ingredients or the His/Bio concentration but do not elaborate further. One laboratory does not list information on the overlays. Three laboratories use media other than Difco. Three laboratories use concentrations different from those recommended by Ames.

PETRI PLATE AGAR

Ames specified Falcon plastic 100 x 15 mm petri plates, and 30 ml of a 1.5% Bacto-Difco agar with 2% glucose and VBME salt solution for the bottom agar. These plates can be stored, according to Ames, for weeks at room temperature. Two laboratories in Table 17 do not discuss their petri plate agar. For most laboratories, the amount of agar in a plate ranges from 20 to 30 ml. Three laboratories indicate a preference for nonethylene oxide sterilized plates. Two laboratories do not use Difco agar. Thirteen laboratories follow the recommendations of Ames for preparation of the agar. One laboratory uses 16 g/l of agar. Four laboratories do not indicate the agar concentration but do give other details. The plates are stored either at room temperature or in the refrigerator.

NUTRIENT BROTH

Ames originally used Difco Nutrient Broth but has since recommended Oxoid #2 broth. Seven laboratories in Table 18 say they use Oxoid #2. Five laboratories use Difco; one uses Columbia Broth, and one recommends Lab-Lemco powder and peptone. The 11 remaining laboratories do not indicate the type of broth used in this laboratory. Storage is indicated by only two laboratories.

TABLE 16. TOP AGAR OVERLAY PREPARATION

Specifications	Reference papers			Laboratories[a,c]					
	Ames	de Serres	Ames revised	Battelle	Bioassay Systems	Biolabs	Biotechnics	EG&G Mason[d]	Genetic Bioassay Branch
Difco agar quantity	0.6%			0.6%	6 g/l	0.6%	0.6%	8 g/l	7 g/l
NaCl quantity	0.5%			0.5%	5 g/l	0.5%	0.5%	5 g/l	5 g/l
Amount dispensed per tube	2 ml	no data available	no data available	2 ml	2 ml	2 ml	2 ml	2.0 ml, 2.5 ml	2 ml
L-Histidine • HCl concentration	0.5 mM			0.5 mM		0.5 mM	0.5 mM	0.5 mM	
Biotin concentration	0.5 mM			0.5 mM		0.5 mM	0.5 mM	0.5 mM	
Quantity His/Bio added[b]	10/100			20/200	1/108	10/100	10/100	10/100	
Melt procedure	steam bath					steam bath	steam bath		microwave or autoclave
Storage	100 ml room temperature			200 ml room temperature	100 ml 4°C	100 ml room temperature	100 ml room temperature	100 ml room temperature	100 ml room temperature

(Continued)

TABLE 16. (Continued)

Specifications	Laboratories								
	Genex	Hilltop	IITRI	Jefferson	Litron	Arthur D. Little	Litton	Monsanto[a]	Microbiological Assoc.
Difco agar quantity	0.6%	6 g/l	0.6%	0.6 g/100 ml	0.6%	0.6%	0.6%	0.6%	
NaCl quantity		5 g/l	0.5%	0.6 g/100 ml	0.5%	0.5%	0.1 M	0.5%	
Amount dispensed per tube	2 ml	2 ml	2 m	2 ml			2 ml	2 ml	2 ml
L-Histidine • HCl concentration	0.5 mM	10 µg/ml agar	0.5 mM	0.5 mM	trace	0.05 ml	0.05 mM	0.5 mM	0.5 mM
Biotin concentration	0.5 mM	12 µg/ml agar	0.5 mM	0.5 mM	trace	0.05 ml	0.05 mM	0.5 mM	0.5 mM
Quantity His/Bio added[b]			10/100	10/100				1/100	10/100
Melt procedure								heat	
Storage								100 ml or 200 ml refrigerate	

(Continued)

TABLE 16. (Continued)

Specifications	Laboratories							
	Meloy	NEIC-EPA[g]	Northrop[h]	Raltech	RTI	SRI	Southwest	Unilab
Difco agar quantity	12 g/2 l[f]		6 g/l	6 g/l	6 g/l	0.6%	6 g/l	0.6%
NaCl quantity	10 g/2 l		5 g/l	5 g/l	5 g/l	0.6%	5 g/l	0.05%
Amount dispensed per tube	2 ml	2 ml	2 ml	2 ml	2.5 ml	2 ml	2 ml	2 ml
L-Histidine • HCl concentration				0.5 mM	48.0 mg/500 ml	0.05 mM	0.5 mM	0.05 mM
Biotin concentration				0.5 mM	61.0 mg/500 ml	0.05 mM	0.5 mM	0.05 mM
Quantity His/Bio added[b]	10/100	20/200		10/100	100/1		10/100	10/100
Melt procedure		autoclave microwave		autoclave			autoclave	steam bath
Storage	100 ml	200 ml refrigerate		100 ml or 400 ml glass serum bottles room temperature 1 yr	glass test tubes 2.5 ml		100 ml room temperature	100 ml room temperature

[a]The following laboratory gives no information on preparation: Hazleton.
[b]Quantity will be expressed as ml His/Bio per ml agar.
[c]Case Western uses Davis Medium with biotin.
[d]In addition, 25 ml H_2O/100 ml are added for a nonactivation assay.
[e]In addition, 25 ml 0.2 M phosphate buffer/100 ml agar are added for a nonactivation assay.
[f]Meloy uses Noble agar.
[g]NEIC-EPA recommends 1.2 g Oxoid #L28 agar/200 ml H_2O and 1 g NaCl/200 ml H_2O.
[h]When preparing histidine-positive agar, 39 mg histidine is used per liter of agar.

TABLE 17. PETRI PLATE AGAR

Specifications	Reference papers			Laboratories[a,b]				
	Ames	de Serres	Ames revised	Battelle	Bioassay Systems	Biolabs	EG&G Mason	Genetic Bioassay Branch
Type of plate	Falcon plastic		Muta-Assay		100 x 15 mm		Muta-Assay	100 x 15 mm
Agar amount/plate	30 ml	no data available			30 ml		25 ml	30 ml
Agar concentration	1.5%			1.5%	1.5%	1.5%		45 g/2655 ml
Glucose concentration	2%			2%	50 ml/l of 40%	2%		200 ml/2655 ml
Salt solution	Vogel Bonner Medium E			VBME	20 ml/l of VBME 50x	VBME		60 ml/2655 ml VBME 50x
Storage	room temperature weeks				500 ml aliquots room temperature			

(Continued)

TABLE 17. (Continued)

Specifications	Laboratories									
	Genex	Hazleton	Hilltop	IITRI	Jefferson	Litron	Arthur D. Little	Litton	Monsanto	NEIC-EPA[e]
Type of plate		100 x 15 mm							non-ethylene oxide sterilized	100 x 15 mm
Agar amount/plate		20 ml		20 ml					25-30 ml	30 ml
Agar concentration	1.5%		15 g/l	1.5%	15 g/l		1.5%		15 g/l	
Glucose concentration	1%		2%	2%	2%	2%	2%	2%	20 g/l	2%
Salt solution	VBM		VBM	VBM 20 ml/l	VBM		VBME	VBME	VBM 20 ml/l	VBME 20 ml/l
Storage		room temperature							4°C	

(Continued)

TABLE 17. (Continued)

Specifications	Laboratories					
	Northrop[f]	Raltech	RTI	SRI	Southwest	Unilab
Type of plate	100 x 15 mm	Muta-Assay	100 x 15 mm		Falcon 100 x 15 mm	Plasta Medic plates
Agar amount/plate	30 ml	30 ml	25 ml		25 ml	30 ml
Agar concentration	15 g/l	15 g	24 g/1500 ml	15 g/l	30 g/1800 ml	1.5%
Glucose concentration	20 g/l	40% 50 ml/l	30 g/1500 ml	10 g/l	20% 100 ml/1800 ml	2%
Salt solution	VBM	VBM 50x 20 ml/l	VBM 30 ml/1500 ml	VBM	VBM 40 ml/1800 ml	VBM
Storage	refrigerator	room temperature weeks	4°C 1 month			

[a]The following laboratories do not give information on petri plate agar: Biotechnics, Microbiological Assoc.
[b]Case Western uses Davis Medium with biotin. Meloy uses Noble agar.
[c]A total of 30 ml His/Bio solution are added to 2655 ml agar solution.
[d]A total of 5 g/l NaCl are added.
[e]Oxoid agar is recommended.
[f]A total of 210 mg/l histidine and 390.4 mg/l biotin are added.

TABLE 18. NUTRIENT BROTH PREPARATION

Specifications	Reference papers			Laboratories[a,b,c]					
	Ames	de Serres	Ames revised	Bioassay Systems	Biolabs	Genetic Bioassay Branch	Monsanto	Meloy	NEIC-EPA[d]
Quantity Nutrient Broth	Difco		Oxoid #2 Broth	8 g/l	8 g/l	8 g/l	8 g/l	8 g/l	
Quantity NaCl		no data available		5 g/l	5 g/l	5 g/l	5 g/l	5 g/l	5 g/l
Quantity dispensed				5 ml			50 ml	10 ml	10 ml
Storage				refrigerator				4°C	

[a]The following laboratories do not give preparation instructions: Battelle, Biotechnics, Genex, Hazleton, Hilltop, Jefferson, Arthur D. Little, Litton, Microbiological Assoc., Northrop, Southwest.

[b]Case Western uses Columbia Broth.

[c]EG&G Mason, IITRI, Litron, Raltech, RTI, SRI, and Unilab use Oxoid #2 broth.

[d]This laboratory uses 10 g/l Lab-Lemco powder and 10 g/l peptone.

SECTION 7

CHEMICAL CONTROLS

CONTROL MUTAGENS/CARCINOGENS ROUTINELY USED[a]

For activation assays Ames recommended using 2-aminofluorene, aflatoxin B_1, and benzo(a)pyrene. de Serres and Shelby recommend using 2-aminoanthracene solely. Fourteen laboratories use 2-aminoanthracene exclusively as shown in Table 19. Two laboratories use 2-aminofluorene exclusively. Two others use 2-aminoanthracene and benzo(a)pyrene. One laboratory uses 2-aminofluorene and aflatoxin B_1, and another uses benzo(a)pyrene, 2-aminoanthracene, and proflavine. Five laboratories do not indicate what chemical they use.

For nonactivation assays using strain TA1535 Ames recommended N-methyl-N-nitrosoguanidine (MNNG) and de Serres and Shelby recommended methyl methanesulfonate (MMS). Six laboratories use MNNG exclusively. Nine laboratories use sodium azide solely. Another uses 1,3-propane sultone for the control for TA1535. One laboratory uses MNNG, MMS, and sodium azide. Another uses sodium azide and 4-fluoro-3-nitrophenyl azide. Still another uses sodium nitrite, N-hexyl-N-nitro-N-nitrosoguanidine. One laboratory uses MNNG, MMS, and 4-Nitroquinoline-N-oxide, and five do not say what chemical they use.

When using TA1537 Ames and de Serres both recommended using 9-aminoacridine. Eighteen laboratories follow this recommendation. Five laboratories do not say what chemical they use. One laboratory uses 4-fluoro-3-nitrophenyl azide, and another uses MNNG and 9-aminoacridine.

For TA1538 Ames suggested 2-aminofluorene and de Serres recommended 4-nitro-O-phenylene diamine, 2-nitrofluorene, and hycanthone methanesulfonate. Thirteen laboratories use 2-nitrofluorene only. Seven do not list the chemical they use. One laboratory uses Daunomycin exclusively, and another uses 2-aminofluorene exclusively. One laboratory uses 2-nitrofluorene and 4-nitro-O-phenylene diamine while one uses 2-nitrofluorene and 4-fluoro-3-nitrophenyl azide. One laboratory uses MNNG and 4-nitroquinoline-N-oxide.

When using TA98 Ames recommended 4-nitroquinoline-N-oxide, Daunomycin, 2-aminofluorene, and aflatoxin B_1. de Serres recommended using 4-nitro-O-phenylene diamine, 2-nitrofluorene, and hycanthone methanesulfonate. Fourteen laboratories use 2-nitrofluorene only. Six laboratories do not list a

[a]Carcinogens/mutagens in Table 19 are placed in categories according to the strains with which they are used as controls.

chemical. One laboratory uses Daunomycin only and one uses 4-nitroquinoline-N-oxide only. One laboratory uses 2-nitrofluorene and hycanthone methanesulfonate. Another uses 2-nitrofluorene and 4-fluoro-3-nitrophenyl azide. Still another uses Daunomycin, MMS, and 4-nitroquinoline-N-oxide.

For strain TA100 Ames suggested MMS, MNNG, 2-aminofluorene, aflatoxin B_1, and 4-nitroquinoline-N-oxide. de Serres' recommendations were sodium azide, MMS, and nitrofurantoin. Nine laboratories use sodium azide only, and three use MNNG only. One laboratory uses 1,3-propane sultone, and one uses MMS; another uses benzo(a)pyrene only. Five laboratories do not list the chemical they use. One laboratory uses MMS and MNNG. One laboratory uses MNNG, MMS, sodium azide, and nitrofurantoin. One laboratory uses sodium azide, 4-fluoro-3-nitrophenyl azide. Another uses MMS and 2-nitrofluorene. Still another uses MNNG, MMS, and 4-nitroquinoline-N-oxide.

PREPARATION OF CONTROL MUTAGENS/CARCINOGENS

Ames recommended the disposable sterile polystyrene screw cap tube as the container of use for carcinogens and listed four possible solvents: H_2O, DMSO, ethanol or p-dioxane. He said carcinogens can be stored in solution in the freezer or refrigerator for extended periods, except for highly reactive compounds which should be made up fresh. de Serres did not recommend storing any compounds in solution.

Fourteen laboratories shown in Table 20 do not discuss their method of preparation. Two laboratories follow Ames' procedure. Five others list only the solvent used. Four laboratories list either their storage container or procedure.

TABLE 19. CONTROL MUTAGENS/CARCINOGENS ROUTINELY USED

Chemical used	Reference papers			Laboratories[a]		
	Ames	de Serres	Ames revised	Battelle	Biolabs	Biotechnics
With S-9	2-Aminofluorene Aflatoxin B_1 Benzo(a)pyrene	2-Aminoanthracene		2-Aminoanthracene	2-Aminofluorene	2-Aminofluorene
Without S-9 TA1535	MNNG[b]	MMS[c] Sodium azide	no data available	Sodium azide		MNNG
TA1537	9-Aminoacridine	9-Aminoacridine		9-Aminoacridine		9-Aminoacridine
TA1538	2-Aminofluorene	4-Nitro-O-phenylene diamine 2-Nitrofluorene Hycanthone methanesulfonate		2-Nitrofluorene		
TA98	4-Nitroquinoline-N-oxide Daunomycin 2-Aminofluorene Aflatoxin B_1	4-Nitro-O-phenylene diamine 2-Nitrofluorene Hycanthone methanesulfonate		2-Nitrofluorene		MMS MNNG
TA100	MMS MNNG 2-Aminofluorene Aflatoxin B_1 4-Nitroquinoline-N-oxide	Sodium azide MMS Nitrofurantoin		Sodium azide		

(Continued)

TABLE 19. (Continued)

Chemical used	Laboratories					
	EG&G Mason	Genetic Bioassay Branch	Genex	Hazleton	Hilltop	IITRI
With S-9	2-Aminoanthracene	2-Anthramine	2-Aminoanthracene	2-Anthramine	2-Anthramine	2-Anthramine
Without S-9 TA1535	1,3-Propane sultone	MNNG MMS Sodium azide	Sodium azide	MNNG	MNNG	Sodium azide
TA1537	9-Aminoacridine	9-Aminoacridine	9-Aminoacridine	9-Aminoacridine	9-Aminoacridine	9-Aminoacridine
TA1538	2-Nitrofluorene	2-Nitrofluorene 4-Nitro-O-phenylene diamine	2-Nitrofluorene	2-Nitrofluorene	2-Nitrofluorene	2-Nitrofluorene
TA98	2-Nitrofluorene	2-Nitrofluorene Hycanthone methanesulfonate	2-Nitrofluorene	2-Nitrofluorene	2-Nitrofluorene	2-Nitrofluorene
TA100	1,3-Propane sultone	MNNG MMS Nitrofurantoin Sodium azide	Sodium azide	MNNG	MNNG	Sodium azide

(Continued)

TABLE 19. (Continued)

Chemical Used	Laboratories					
	Jefferson	Litron	Arthur D. Little	Litton	Monsanto	Microbiological Assoc.
With S-9	2-Anthramine	Benzo(a)pyrene 2-Aminoanthracene Proflavine	2-Anthramine	2-Anthramine	2-Aminoanthracene Benzo(a)pyrene	2-Aminoanthracene
Without S-9 TA1535	Sodium azide	Sodium azide 4-Fluoro-3-nitrophenyl azide	MNNG	Sodium azide	$NaNO_2$[d] N-Hexyl-N-nitro-N-nitrosoguanidine	Sodium azide
TA1537	9-Aminoacridine	9-Aminoacridine 4-Fluoro-3-nitrophenyl azide	9-Aminoacridine	9-aminoacridine	9-Aminoacridine	9-Aminoacridine
TA1538	2-Nitrofluorene	2-Nitrofluorene 4-Fluoro-3-nitrophenyl azide	Daunomycin	2-Nitrofluorene		2-Nitrofluorene
TA98	2-Nitrofluorene	2-Nitrofluorene 4-Fluoro-3-nitrophenyl azide	Daunomycin	2-Nitrofluorene	2-Nitrofluorene	2-Nitrofluorene
TA100	Sodium azide	Sodium azide 4-Fluoro-3-nitrophenyl azide	MMS	Sodium azide	MMS 2-Nitrofluorene	Sodium azide

(Continued)

TABLE 19. (Continued)

Chemical used	Laboratories					
	Meloy	MEIC-EPA	Northrop	Raltech	RTI	SRI
With S-9	2-Aminoanthracene Benzo(a)pyrene	2-Aminofluorene Aflatoxin B_1	2-Anthramine		2-Anthramine	2-Aminothracene
Without S-9 TA1535	MNNG	MNNG MMS 4-Nitroquinoline-N-oxide	Sodium azide	MNNG	Sodium azide	Sodium azide
TA1537	9-Aminoacridine	9-Aminoacridine MNNG	9-Aminoacridine	9-Aminoacridine	9-Aminoacridine	9-Aminoacridine
TA1538	2-Nitrofluorene	MNNG 4-Nitroquinoline-N-oxide	2-Nitrofluorene	2-Aminofluorene	2-Nitrofluorene	2-Nitrofluorene
TA98	2-Nitrofluorene	Daunomycin 4-Nitroquinoline-N-oxide MMS	2-Nitrofluorene	4-Nitroquinoline-N-oxide	2-Nitrofluorene	2-Nitrofluorene
TA100	MNNG	MNNG MMS 4-Nitroquinoline-N-oxide	Sodium azide	Benzo(a)pyrene	Sodium azide	Sodium azide

[a]The following laboratories do not indicate the control chemicals used: Bioassay Systems, Case Western, Southwest, Unilab.
[b]MNNG: N-methyl-N-nitroaoguanidine.
[c]MMS: Methyl methanesulfonate.
[d]$NaNO_2$: Sodium Nitrite.

TABLE 20. PREPARATION OF CONTROL MUTAGENS/CARCINOGENS

Specifications	Reference papers			Laboratories[a]					
	Ames	de Serres	Ames revised	Battelle	Bioassay Systems	Biolabs	IITRI	Jefferson	Arthur D. Little
Container	disposable sterile polystyrene screw cap tubes				disposable sterile polystyrene screw cap tubes	sterile polystyrene screw cap tubes	pyrex glass tubes		
Solvents	H_2O DMSO ETOH p-Dioxane		no data available	DMSO	H_2O DMSO	H_2O DMSO ETOH p-Dioxane	H_2O DMSO	DMSO	DMSO ETOH
Storage	freezer -20°C or refrigerator extended periods	should not be stored			-20°C extended periods[b]	-20°C[b]			

(Continued)

TABLE 20. (Continued)

Specifications	Laboratories				
	Litton	Monsanto	NEIC-EPA	Raltech	Unilab
Container		Sterile septum crimped vials			disposable plastic test tubes
Solvents	DMSO H_2O ETOH		DMSO	DMSO	
Storage		room temperature aluminum foil covered vials 6-8 weeks	6 months for some	freezer -90°C	spill tray

[a]The following laboratories give no specifications for control preparation: Biotechnics, Case Western, EG&G Mason, GBB, Genex, Hazleton, Hilltop, Litron, Microbiological Assoc., Meloy, Northrop, RTI, SRI, and Southwest.
[b]Highly reactive compounds should be made up fresh.
[c]DMSO: dimethylsulfoxide; ETOH: ethanol.

SECTION 8

ASSAY PROCEDURE

Ames recommended the following procedure for the assay. An aliquot of 0.1 ml from a 10^8 culture of Salmonella is added to a tube containing 2 ml molten top agar at 45°C, followed by the sample to be tested and 0.5 ml of S-9 mix. The tube is rotated between the palms to mix the contents and, within 20 sec, poured onto a petri plate. The plates are left to harden for one hour and then incubated in the dark for two days at 37°C.

de Serres and Shelby recommended a culture of 1 or 2 x 10^9 concentration of Salmonella and an extension of the incubation time from 48 h to 48-72 h. They suggested duplicate plating as the minimum with triplicate plating preferred, and used aliquots of 0.2 µg to 5 mg of sample. They recommended both that sterility controls include the solvent and S-9 mix, and that spot checked phenotype monitoring be performed.

The most variation between the laboratories shown in Table 21 comes in the order in which constituents are added to the top agar. Half the laboratories indicate a preference to the order of addition: Salmonella, sample, and S-9. The other laboratories use different permutations or do not list an order. Temperatures for top agar vary from 43 to 56°C. Incubation times range from 36 to 72 h. Duplicate or triplicate plating is recommended.

Five laboratories substitute the volume taken up by S-9 by using KCl, saline, or buffer in a nonactivation assay. Three laboratories place their plates in sealed bags for the incubation time.

Ames has revised his mixing technique: he now uses a vortex at low speed. Eight laboratories mention using a vortex in their protocols.

Three laboratories indicate use of only a preincubation technique with the Salmonella and sample.

TABLE 21. ASSAY PROCEDURE

Specifications	Reference papers			Laboratories			
	Ames	de Serres	Ames revised	Battelle	Bioassay Systems	Biolabs	Biotechnics[a]
Quantity top agar per tube	2 ml			2 ml	2 ml	2 ml	2 ml
Temperature top agar	45°C			45°C	49°C	45°C	
Quantity Salmonella per tube	0.1 ml of a 10^8 culture	1-2 x 10^9 culture		0.1 ml overnight culture	0.1 ml overnight culture	0.1 ml overnight culture	0.1 ml overnight culture
Quantity S-9 mix per tube	0.5 ml			0.2 ml 10% mix	0.5 ml	0.5 ml	0.5 ml
Order of addition 1.	Salmonella				sample	Salmonella	Salmonella
2.	sample				Salmonella	sample	sample
3.	S-9			S-9	S-9	S-9	S-9
Mixing technique	rotate between palms		vortex low speed		rotate between palms several minutes	rotate between palms	rotate between palms several minutes
Hardening time	1 h						
Incubation	37°C dark 2 days	37°C 48-72 h		72 h	37°C 2 days in bags		37°C dark 2 days
Replications		duplicate as minimum		duplicate	duplicate	triplicate	
Number of dose ranges				5		3-5	5
Sterility controls		Solvent S-9 mix		S-9 mix sample solvent			

(Continued)

TABLE 21. (Continued)

Specifications	Laboratories							
	Case Western[a,b]	EG&G Mason[c]	Genetic Bioassay Branch	Genex	Hazleton	Hilltop	IITRI	Jefferson[d]
Quantity top agar per tube	2 ml	2.0 ml or 2.5 ml	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml
Temperature top agar		45°C	45°C			45°C	45°C	46°C
Quantity Salmonella per tube	0.1 ml of 1:20 dilution	50 µl	0.1 ml	0.05 ml overnight culture	0.1 ml overnight culture	0.1 ml 24-h culture	0.1 ml	0.1 ml
Quantity S-9 mix per tube	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Order of addition 1. 2. 3.	Salmonella sample S-9 top agar	Salmonella sample S-9	Salmonella S-9 sample		Salmonella sample S-9	sample Salmonella S-9	Salmonella sample S-9	sample S-9 Salmonella
Mixing technique		vortex	vortex	vortex			rotate between palms	
Hardening time					1 h			
Incubation	37°C 48 h	37°C 48 h	37°C 72 h	37°C 3 days	37°C ± 0.5°C dark 2 days	37°C 48-56 h	37°C 2 days	37°C 72 h
Replications		triplicate	triplicate	duplicate	triplicate		triplicate	duplicate
Number of dose ranges		5	5	5	5	5	5	
Sterility controls			sample S-9				mutagens plates S-9 sample overlay	

(Continued)

TABLE 21. (Continued)

Specifications	Laboratories							
	Litron	Arthur D. Little	Litton[b]	Monsanto[c]	Microbiological Assoc.	Meloy[b]	NEIC-EPA	Northrop
Quantity top agar per tube			2 ml	2 ml	2 ml	2 ml	2 ml	2 ml
Temperature top agar			48-45°C	45°C		45°C	45°C	46°C
Quantity *Salmonella* per tube	0.1 ml of a 10^8 culture		0.1 ml-0.2 ml	0.1 ml of a 5×10^8 or 10^9 culture	0.1 ml overnight broth	0.1 ml	0.1 ml	0.1 ml of a 2×10^8 culture
Quantity S-9 mix per tube	0.5 ml		0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Order of addition 1. 2. 3.			sample *Salmonella* S-9	sample *Salmonella* S-9	*Salmonella* sample S-9	*Salmonella* sample S-9	*Salmonella* sample S-9	*Salmonella* sample S-9
Mixing technique			swirl	mix rapidly	vortex	vortex	vortex	vortex
Hardening time								
Incubation	37°C 48-60 h		37°C 36-72 h	37°C 48 h in bags	37°C ± 2°C 46-48 h	37°C 48 h in bags	37° 48 h in bags	37°C dark 48-72 h
Replications	duplicate	duplicate		triplicate	triplicate	duplicate	duplicate	duplicate
Number of dose ranges	6		6	6	5	5	5	
Sterility controls		mutagens sample S-9 solvent			sample S-9	solvent media S-9 sample	S-9 sample	S-9 sample

(Continued)

TABLE 21. (Continued)

Specifications	Laboratories				
	Raltech	RTI[9]	SRI	Southwest	Unilab
Quantity top agar per tube	2 ml	2.5 ml	2 ml	2 ml	2 ml
Temperature top agar			43°C	45°C	45°C
Quantity *Salmonella* per tube	0.1 ml of a $2\text{-}4 \times 10^8$ culture		0.05 ml	0.1 ml	0.1 ml
Quantity S-9 mix per tube	0.5 ml		0.5 ml	0.5 ml	
Order of addition 1.	*Salmonella*	S-9	*Salmonella*	*Salmonella*	
2.	sample	*Salmonella*	S-9	sample	
3.	S-9	sample	sample	S-9	
Mixing technique	vortex	shake	stir	vortex 2 sec	
Hardening time	1/2 h			30 min	
Incubation	37°C dark 48-72 h		37°C 2 days	72 h	37°C 72 h
Replications	duplicate or triplicate	triplicate		triplicate	
Number of dose ranges	5		6	7	
Sterility controls	S-9 sample solvent media	all components		media solvent S-9	

[a]Preincubate for 20 minutes.
[b]In addition, 0.5 ml buffer is added for a nonactivation assay.
[c]A total of 2.5 ml top agar is used for a nonactivation assay rather than 2.0 ml.
[d]In addition, 0.5 ml of 0.15 M KCl is added for a nonactivation assay.
[e]In addition, 0.5 ml of buffer or saline is added for a nonactivation assay.
[f]Bacteria is spundown and resuspended.
[g]RTI uses an NADPH generating system; for details, see their protocol.

SECTION 9

SAFETY PRECAUTIONS

Safety precautions were not generally found in the submitted protocols. Only seven laboratories devoted sections to safety.[a]

Two of these laboratories include a separate safety manual in their protocols. Other laboratories include sections which discuss general precautions for dealing with carcinogens.

Some of the more important topics mentioned in these sections include: storage, weighing, and disposal of carcinogens; spill clean-up procedures; protective clothing requirements; laboratory access; and medical surveillance of personnel.

Since safety protocols were not requested with the Ames' Test Protocols, it would be unfair to suggest that these are the only laboratories with safety standards. The submitted safety sections do, however, delineate the areas of concern when considering potential laboratory health hazards.

[a] Protocols with references to safety came from Genetic Bioassay Branch, Meloy, Litton, Northrop, NEIC-EPA, Raltech, and Southwest.

SECTION 10

DISCUSSION

As a first step in establishing Quality Assurance/Quality Control (QA/QC) procedures for *in vitro* bioassays, the Ames *Salmonella* plate incorporation bioassay protocols from 25 laboratories were compared. At this stage, no attempt was made to evaluate a protocol or to designate a preferred method; however, the authors hope that this effort will help each laboratory evaluate its own protocols and standard operating procedures (SOP's). Since many of these laboratories may maintain highly detailed SOP's in addition to the protocols, the protocols do not necessarily reflect the competence of the submitting laboratory. Therefore, inclusion is neither an attempt to criticize nor endorse any procedure or laboratory. It is, however, an attempt to understand what differences in operating procedures do exist.

A list of 46 laboratories performing this bacterial assay was obtained from Dr. Mike Shelby, National Institute of Environmental Health Sciences. Thirty-three of the 46 laboratories (71.7%) responded and supplied 25 protocols (54.3%) that were made available for compilation and comparison. These protocols are published in a companion EPA report entitled, "Compilation of Ames *Salmonella typhimurium* Plate Incorporation Test Protocols." The present document is an attempt to perform an informative comparison of the 25 available protocols. No attempt was made to compile or compare additionally submitted materials such as procedures for preincubation, suspension tests, etc.

Table 22 summarizes 24 identifiable procedures and/or preparations associated with this bioassay and how many laboratories described and/or adhered to or modified the 1975 Ames, *et al.*, protocol. In general, most laboratories follow most of the procedures as outlined by Ames; however, it appears that each laboratory deviates from this standard protocol in some manner. There was strong concordance with Dr. Ames on the selection of tester strains, the preparation of the S-9 mix, and the preparation of the microsomal salt solution. In many cases, the deviations were minor: e.g., allowing the overlaid plates to harden for 30 minutes instead of the recommended hour before inverting in the incubator.

Most laboratories differ from the Ames, *et al.*, 1975 protocol in the selection of some control compound and in the actual assay exposure procedure. Again, most of these modifications would be expected to have minor or negligible effect upon any qualitative results obtained. Only one laboratory prescribed the routine verification of compound sensitivity for strains TA98 and TA100. Most procedures and media preparation instructions were left out

of one or more protocols; however, a laboratory SOP may have included these specific instructions.

Conparison of current protocols may improve both individual experimental procedure and the consistency of results from different laboratories. In addition, this study may help identify monitorable parameters within the test procedures so that adequate QA/QC procedures can be established.

TABLE 22. INCLUSION OF PROCEDURES AND PREPARATION IN 25 AMES *SALMONELLA* PLATE INCORPORATION PROTOCOLS

Procedure/Preparation	Described Procedure				Mentioned but Not Described		No Procedure Given		Procedure not Performed	
	Ames		Modified/Partial							
	No.	%	No.	%	No.	%	No.	%	No.	%
Bacterial strains routinely used	20	80	4	16	0	0	1	4	0	0
Storage of strains	6	24	0	0	0	0	19	76	0	0
Histidine requirement	9	36	2	8	4	16	10	40	0	0
Determination of rfa	9	36	5	20	5	20	6	36	0	0
Ampicillin resistance	3	12	9	36	6	24	6	36	1	4
uvrB Deletion verification	8	32	4	16	5	20	8	32	0	0
Compound sensitivity verification	1	4	0	0	0	0	24	96	0	0
Spontaneous reversion rate	11	44	0	0	5	20	9	36	0	0
S-9: Induction of Animal	9	36	8	32	5	20	3	12	0	0
S-9: Preparation	8	32	13	52	0	0	4	16	0	0
S-9: Storage	18	72	0	0	0	0	7	28	0	0
S-9 mix: Preparation	22	88	2	8	0	0	1	4	0	0
Sodium phosphate buffer	10	40	4	16	9	36	2	8	0	0
Microsomal salt solution	21	84	3	12	0	0	1	4	0	0
Glucose stock solution	a	...	20	80	0	0	5	20	0	0
His/Bio stock solution	14	56	9	36	0	0	2	8	0	0
VBME salt solution	a	...	15	60	0	0	10	40	0	0
Top agar overlays	9	36	15	60	0	0	1	4	0	0
Petri plate agar	13	52	10	40	0	0	2	8	0	0
Nutrient broth (Difco)	5	20	9	36	0	0	11	44	0	0
Control compounds	1	4	20	80	0	0	4	16	0	0
Preparation of controls	2	8	9	36	0	0	14	56	0	0
Assay procedure	5	20	20	80	0	0	0	0	0	0
Safety procedures	a	...	7[b]	...	0	...	18[b]	...	0	0

[a]Not discussed in paper by Ames, *et al.*, 1975.
[b]Safety protocols were not requested, but were submitted by some laboratories.

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